A STUDY OF THE MUTUAL INTERACTION OF SOME ANTIBIOTICS AND NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN VITRO

by

AMINA ASGHAR

059-GCU-PhD-CHEM-2008

DEPARTMENT OF CHEMISTRY

GC UNIVERSITY, LAHORE

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A STUDY OF THE MUTUAL INTERACTION OF SOME ANTIBIOTICS AND NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN VITRO

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by

AMINA ASGHAR

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GC UNIVERSITY, LAHORE

RESEARCH COMPLETION CERTIFICATE

Certified that the research work contained in the thesis "A study of the mutual interaction of some antibiotics and non-steroidal anti-inflammatory drugs *in vitro"* has been carried out and completed by Miss Amina Asghar, Reg. No. 059-GCU-PhD-Chem-2008 under our supervision during her PhD (Chemistry) studies. The quantum and the quality of the work contained in this thesis are adequate for the award of degree of Doctor of Philosophy.

Date: _____

Prof. Dr. Mohammad Saeed Iqbal	Prof. Dr. Islam Ullah Khan
Supervisor	Co-Supervisor

Submitted through

Prof. Dr. Islam Ullah Khan Chairman Department of Chemistry, GC University, Lahore	Controller of Examination GC University, Lahore

DECLARATION

I Amina Asghar Reg. No. 059-GCU-PhD-CHEM-2008 a student of PhD in the subject of Chemistry, Session: 2008-2014, hereby declare that the matter printed in the thesis titled: "A Study of the Mutual Introduction of Some Antibiotic and Non-Steroidal Anti-Inflammatory Drugs in Vitro" is my own work and has not been printed, published and submitted as research work or thesis in any form in any university or research institution etc. in Pakistan or abroad.

Date: _____ _____

Amina Asghar

DEDICATION

Affectionately dedicated

to

my loving parents

Acknowledgements

In the name of Allah almighty, the most beneficent the merciful. All praise to Him who blessed me with knowledge, courage and strength to work and to his last prophet (peace be upon him) for whom this wide and huge universe was created and who is the pioneer of education and research. Being his spiritual follower and believer, I tried to get educated and explore the secrets of universe.

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AMINA ASGHAR

Abstract

This work aimed at improving efficacy and reducing toxicity of non-steroidal anti-inflammatory (NSAIDs) and anti-bacterial drugs by designing and synthesizing mutual prodrugs with dual activities. The NSAIDs were ibuprofen, flurbiprofen and aspirin, which contained a carboxylic group as part of their structure. The antibacterial included ampicillin, metronidazole, isoniazid, sulfamethoxazole, sulfamerazine, sulfamethazine, sulfanilamide, 7-ADCA and 7-AVCA, which contained an amino group as part of their structure. In the prodrugs of these compounds the two drugs were covalently linked together forming an amide linkage. In addition to these a prodrug from benzydamine, containing amino group, and cefazoline, containing carboxylic group was synthesized, in which the two drugs formed a quaternary ammonium salt. All the synthesized compounds were characterized by use of diverse analytical techniques including elemental analysis, FT-IR, electronic spectra, ^{1}H and ^{13}C NMR, ESI-MS and single crystal XRD techniques.

The new compounds were subjected to anti-bacterial, anti-inflammatory, enzyme inhibition and toxicity tests in order to evaluate them as more effective and safe drugs with dual activities. Some of the activity related properties, which could not be determined experimentally, were determined through computational analysis. The results showed that aspirin, flurbiprofen and ibuprofen prodrugs perform better (having moderate to significant difference) than the parent drugs in anti-bacterial and anti-inflammatory tests. The computational analysis also suggests that the prodrugs possess better druglike properties and bioavailability with slight variations. Thus this study clearly indicates that mutual prodrug is an advantageous option where a concomitant treatment is required.

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1. Introduction

1.1. General

Generally infections are associated with some kind of inflammation; as a result it is standard practice to prescribe anti-infective and anti-inflammatory drugs concomitantly. So it is desirable to have drugs with anti-infective and anti-inflammatory activities embodied in one molecule. Very few drugs are available having such a dual activity. One approach to achieve this end could be to synthetically combine appropriate anti-infective with anti-inflammatory molecules. Such combinations may provide us with the so-called mutual prodrugs for use in a single dose. The present work was planned to study the formation of diverse combination products by use of various anti-infective and anti-inflammatory agents, which may act as mutual prodrugs. Several studies involving synthesis of mutual prodrugs have been reported but there exists only a few reports describing the synthesis of prodrugs from anti-infective and anti-inflammatory agents. A brief review of literature in this regard is presented in the following paragraph.

The mutual prodrugs, synthesized and reported in the literature, mainly focused to mask the free carboxylic acid group of the anti-inflammatory drugs with a view to improve taste or reduce ulcerogenic and hepatotoxic effects [1]. Drugs used for synthesizing these specific mutual prodrugs were ibuprofen, flurbiprofen, naproxen, diclofenac, 4-aminophenylacetic acid, 5-aminosalicylic acid among the NSAIDs, while sulfa drugs and other antibiotics were modified at terminal amino groups [2**,** 3**,** 4**,**5]**.**

The objective of the present work was to design and synthesize mutual prodrugs involving various antibacterial and NSAIDs. The new compounds were expected to be less toxic and retain their pharmacological activity with some advanced features.

1.2. Prevalence of infections and Inflammation

It can be assessed through a simple survey of patients reporting to hospitals and general physicians that infections and inflammatory response thereof are the most prevalent all over the world. The NSAIDs and anti-infective enjoy about 30% share in drug market. Table 1 lists a pattern of drug usage [6].

Infection is a response of a host tissue to the attack of microorganisms on body. Infection can be caused by infectious agents such as viruses, prions, viroids, and microorganisms. Immune system of the host organisms fights against infections through inflammatory response. Thus inflammation is a defensive attempt to remove injurious stimuli and to start the healing process and itself is not a replacement of infection". The most common infections resulting in inflammation (fever, pain, loss of function and swelling) in Pakistan are listed in Table 2.

1.3. Antibiotics

Antibiotics are mainly the metabolic products of microorganisms, which can inhibit the growth of other microorganisms or kill them [7]. They can be classified, according to their mechanism of action, into four types.

i) Antibiotics which inhibit biosynthesis of bacterial cell wall [8**,** 9]: Cephalosporins (ampicillin, 7-amino deacetoxycephalosporanic acid 7-amino-3-vinylcephalosporanic acid, amoxicillin) [10], glycopeptides, lipoglycopeptides [11] and isoniazid [12] are some of the examples.

Table 1: *Most commonly used drugs*

		0.6 Years (*n=775)*		7.11 Years (*n=284)*		≥ 12Years (*n=127)*		Male (*n=665)*		Female (*n=521)*		Inpatient (*n=541)*		Outpatient (*n=845)*	
Drugs	%	Drug	%	Drug	%	Drug	%	Drug	%	Drug	%	Drug	%	Drug	%
1	12.6	1	15.1	2	12.3	4	7.9	1	5.5	1	13.5	1	19.8	2	8.9
3	4.7	3	6.2	5	4.6	1	5.5	5	2.3	3	5.4	2	6.2	6	5.1
5	3.5	9	3	4	3.5	14	3.9	4	4.2	4	4	18	3.0	7	4.5
8	2.3	12	2.6	8	2.5	2	3.1	9	2.7	7	2.1	8	2.8	13	2.8
10	2	8	2.3	14	2.1	17	2.4	14	2	20	3.0	10	2.1	21	2.0

1: Paracetamol, 2: Salbutanol, 3: augmentin, 4: Ibuprofen, 5: Folic Acid, 6: Fluticasone, 7: Mometasone, 8: Azithromycine, 9: Rehydration Salt, 10: Chlorpheniramine,, 13: Symbicot turbuhaler, 14: Diclofenac Get, 18: cefuroxime 20: Ceftriaxone, 21: sodium Valporate

Table 2: *Most common infections and inflammatory responses observed in Pakistan*

Infection	Inflammatory response	Infection	Inflammatory response
Respiratory tract		*Skin and soft tissue*	
Pharyngitis, laryngitis sinusitis	Fever	Boils, abscesses	Swelling, irritation and pain
Bronchitis	Headache	Diabetic foot infection	Foot pain
Pneumonia	Fever + Chest pain	*Urinary tract*	
Tuberculosis	Fever + Lymphadenitis	Cystitis, urethritis	Flank along with fever and abdomen pain
ENT		*Gastrointestinal tract*	
Otitis Media	fever, ear pain along with feeling of fullness	Amoebic dysentery	Pain in stomach Lose motions
Tonsillitis	Fever, Throat ache	Typhoid fever	Fever

ii) Antibiotics that inhibit protein biosynthesis [13]: Examples are: aminoglycoside, tetracycline, macrolides, lincosamides, streptogramins and oxazolidinones.

iii) Antibiotics that inhibit DNA/RNA" synthesis [14,15]: These include quinolones and rifamycins.

iv) Antibiotics that inhibit folate synthesis [16**,** 17]: Sulfonamides belong to this group .

1.4. Resistance to antibiotics

Antibiotics are great tools to fight infections; but their unnecessary/excessive use has resulted into such microorganisms which show antimicrobial resistance for the medicines to which originally these were sensitive. Antibiotic resistance is a serious and rapidly growing problem; World Health Organization has declared antibiotic resistant organisms as nightmare bacteria. This issue of drug resistance is getting serious day by day not only in developing but also developed countries. Antibiotics usually have a particular target and specific mode of action, thus these interact with a microbe similar to a lock and key model. Antibiotics bond to specific parts of bacteria, and either impair or kill them. A specific key (the right antibiotic molecule) is necessary to fit in the specific lock (a receptor molecule on the target bacterium). In the evolutionary competition between antimicrobials and the bacteria, the bacteria evolve ways of fighting back. They have natural process of occasional changes to their DNA. These genetic changes lead to the development of new traits, some of which allow the individual bacterium to counteract the effects of the antibiotic substance. Antibiotics, which were successfully being used for treatment of infectious diseases, stopped working against antibiotic resistant bacteria as result of mutation in

their DNA. Once the resistance develops in bacteria, these resistant species can share and transfer genetic information to other non-resistant bacteria, hence making them resistant too. As a result some "superbugs" (resistant to all available antibiotics) [18] have come into being.

1.5. New generations of antibiotics

As a result of this ever-increasing resistance problem in bacteria, the antibacterial have limited life span and the resistant drugs are being replaced with the new analogues, generally known as new generations

The new generations of antibacterial are commonly designed by substituting different groups on a pharmacophore. If the substituent is another active drug, the so-called mutual prodrugs may be obtained. Such analogues would possess unique properties with less exposure of body to the unwanted substituents in the conventional prodrugs. New generations of different antibiotics are being synthesized through structural modifications. Cephalosporins of the 1^{st} generation were effective against haemophilus and other Gram-positive bacteria. This was followed by development of 2^{nd}, 3^{rd} and 4^{th} generations [19]. Similarly quinolones, also, have been divided into generations based on their antibacterial spectrum [20]. Research on new generations is vital to address this new threat of getting into a post-antibacterial era.

1.6. Anti-inflammatory drugs

Any chemical substance that reduces/treats inflammation is known as anti-inflammatory drug. Anti-inflammatory drugs can broadly be divided into two classes, i. e., steroidal and non-steroidal.

1.6.1. Steroidal anti-inflammatory drugs

There are a number of steroids which can reduce inflammation or swelling by binding to glucocorticoid receptors [21]**.** These drugs are also known as corticosteroids. The other type, immune selective anti-inflammatory steroids, is a class of peptides [22**]**. Steroidal therapy sometimes results in irreversible adverse effects [23]. This problem led the medicinal chemists to discover NSAIDs [24].

1.6.2. Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs are non-narcotic in nature. These reduce inflammation along with pain by inhibiting cyclooxygenases, COX-1 and COX-2, [25**,** 26]. The COX-2 synthesizes prostaglandins, which cause inflammation, whereas, the COX-1 helps in platelet formation and also act on parietal cells to protect stomach. NSAIDs can be classified into 8 broad categories on the basis of their chemical structure as: i) arylacetic acid analogues, ii) hetero arylacetic acid analogues, iii) arylpropionic acid analogues, iv) naphthalene acetic acid analogues, v) salicylic acid analogues, vi) pyrazolones, vii) pyrazolodiones and viii) miscellaneous anti-inflammatory drugs.

The great chemical diversity of NSAIDs yields a big range of pharmacokinetic characters. Although there are a number of differences in the kinetics of NSAIDs yet there are some common properties. All of the NSAIDs with few exceptions (Naphthalene acetic acid derivative) are weak organic acids.

1.7. Drug interactions

Co-administration of the two different types of drugs has resulted in surprising and unpredicted effects in the patients. This is probably due to altered pharmacokinetics of

the one or both the drugs [27]. These interactions are commonly referred to as drug interactions [28, 29, 30, 31, 32, 33, 34]. What makes it worse is the large numbers of drugs that are being introduced every year and thus new interactions are increasingly reported. Such interactions may suppress the effectiveness of one of the two drugs, cause unexpected side effects [35, 36] or increase the action of a particular drug [37, 38, 39, 40, 41] so these can be synergistic (where the drug's action is increased) or antagonistic (when the drug's action is decreased [42, 43] or a completely new effect may be produced that neither produces on its own. [44, 45]. Some drug interactions can even be harmful .These drug interactions may occur out of any accidental misuse of medicines due to lack of knowledge about the active ingredients [46, 47]. These processes may include alterations in the pharmacokinetics of the drug [48 ,49 ,50, 51, 52] such as alterations in the absorption, distribution, metabolism, and excretion of a drug [53, 54, 55, 56]. These may be classified as chemical and biological interactions [57].

1.8. Prodrugs

Prodrugs are the attempts towards decreased toxicity and improved bioavailability in drugs. These are apparently inactive molecules by themselves and produce active metabolites in the body. In prodrugs, an enzymatic or a chemical transformation is required to release an active drug. Prodrugs are generally designed to make the intended drug safer with increased solubility to enhance oral bioavailability, decreased toxicity, improved chemical stability, avoid premature metabolism, adequate tissue penetration and to improve taste [58]. The key role of a prodrug is to mask a polar functional group (–XH) where X can be –OH, –COOH, –SH, and –NH

in a transient manner so that once the prodrug is on the target site, it hydrolyses to release the active drug molecule [59].

The prodrug approach in drug design is relatively a new and versatile method, applied to a wide range of drug formulations. However, for prodrug strategies to be successful, analysis of parent drug properties and the proper identification of possible barriers are important. Clinically, the majority of prodrugs are used with the aim of enhancing drug permeation by increasing lipophilicity. Prodrugs provide opportunities to overcome problems to drug formulation and delivery such as chemical instability, insufficient oral absorption, poor aqueous solubility, lack of site specificity, rapid pre-systemic metabolism, inadequate brain penetration, toxicity and local irritation [60]. Various prodrugs have been designed and developed to overcome various barriers to drug utilization [61, 62].

The prodrugs are designed in a way to directly attach the intended drug to a carrier group. Prodrugs can also be attached to the carrier group through a linking group. An ideal prodrug should possess following properties [63].

i) Pharmacological inertness;

ii) Rapid transformation into the active form at the target site;"

iii) Non-toxic metabolic fragments followed by their rapid elimination".

Additional benefits of prodrugs thus include: additional biological action; improved pharmacokinetics [64]; reduced side effects [65, 66, 67, 68]; increased bioavailability [69]; better stability [70]; reduction in dose and synergistic effect [71].

Prodrug approach has been a preferred research area amongst the drug design scientists in last decade. This area has a wide range of applications [72, 73, 74].

Prodrug of NSAIDs [75, 76, 77, 78, 79, 80, 81, 82, 83] and antibiotics [84] have been reported. But there are only a few reports of synthesis of mutual prodrugs.

1.9. Limitation of prodrug design

Prodrug design has proven extremely valuable in overcoming various undesirable properties of drugs. On the other side, it can also give rise to a large number of newer difficulties, mainly in the assessment of pharmacological, pharmacokinetic, toxicological and clinical properties of prodrugs [85]. Ideally, the design of a suitable prodrug structure should be considered at the early steps of preclinical development, keeping in mind that prodrugs can alter the tissue distribution, bioavailability, efficacy and the toxicity of the parent drug. Several important elements should be carefully examined while designing a prodrug structure. The absorption, distribution, metabolism, excretion and pharmacokinetic properties need to be comprehensively understood [86].

1.10. Mutual prodrugs

In this class of prodrug, prodrug consists of two pharmacologically active molecules can be attached together where each acts as a promoiety for the other and vice versa [87,88]. A mutual prodrug is a bipartite or tripartite prodrug in which the carrier is a synergistic drug to the intended drug. Benorylate is a mutual prodrug of aspirin and paracetamol. A prodrug relies upon change within the body to deliver the parent active drug to produce its pharmacological effect. The major drawback of the prodrug approach is the promoiety, which is basically an unwanted part, which when released can result in adverse effects. The term prodrug/co-drug refers to two or even more therapeutic compounds active against the same or different diseases and are bonded

through a covalent chemical linkage. Advantages include synergistic modulation of the process, enhancement of drug delivery, pharmacokinetic properties and the potential to enhance stability by masking labile functional groups. The amount of published work on co-drugs is limited but the available data suggest the mutual prodrug concept could provide a significant therapeutic improvement in dermatological diseases.

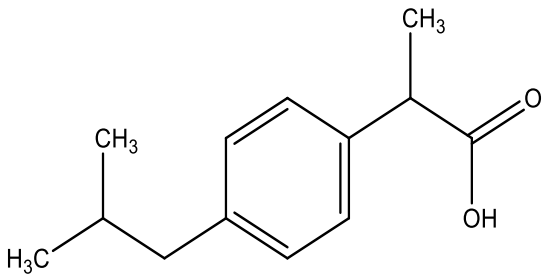
1.11. Drugs selected for present work

1.11.1. NSAIDs

Most commonly used NSAIDs including ibuprofen, flurbiprofen, aspirin and benzydamine hydrochloride were selected for this work.

Ibuprofen

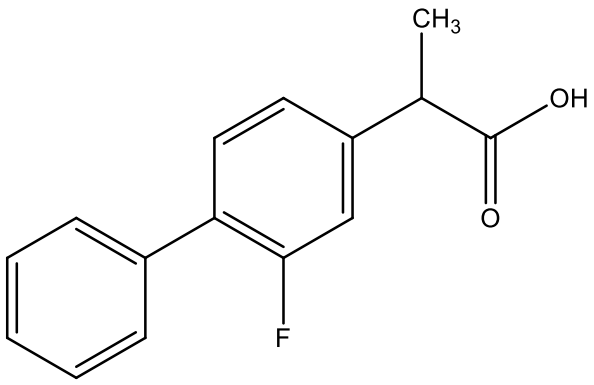
Ibuprofen, 2-(4-isobutylphenyl) propionic acid, is a phenyl propionic acid (structure given below) and is in use for relieving pain (muscle ache, headache, toothache and backache), common cold, soreness, swelling and stiffness caused by arthritis [89]. Ibuprofen is also used to reduce fever and inflammation. It is most frequently prescribed NSAID. In lower dose, it works as a non-narcotic analgesic." It is soluble in methanol, dimethyl sulfoxide and dichloromethane



Ibuprofen

Flurbiprofen

Flurbiprofen, 2-(2-fluoro-[1,1'-biphenyl]-4-yl)propanoic acid (structure given below) is a propionic acid. It is not only available as oral medicine but also as a topical ophthalmic formulation [90]. Flurbiprofen is effective intravenously for preoperative analgesia in minor surgery (neck, ear and nose) and also in treatment of sore throat. It is soluble in acetonitrile and dimethyl sulfoxide.



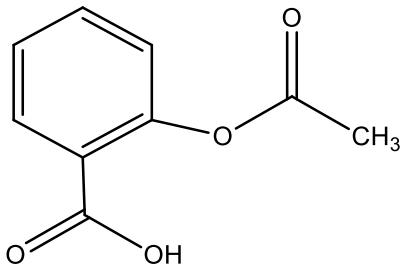
Flurbiprofen

Ibuprofen and Flurbiprofen both have gained acceptance in the treatment of osteoarthritis [91]. These are well absorbed on oral administration, undergo hepatic metabolism and excreted through kidney.

Aspirin

The most commonly employed NSAID, aspirin, 2-acetoxybenzoic acid (structure given below), is the prototype of the NSAIDs. Aspirin was officially approved by FDA in 1939. This is the standard anti-inflammatory drug for all other NSAIDs. It is a weak organic acid and is unique among the other NSAIDs as it irreversibly acetylates cyclooxygenase. Aspirin has not only anti-inflammatory but also antipyretic and analgesic effect. It has three therapeutic actions and these are reducing inflammation, pain and fever. This mainly works by inhibiting prostaglandin synthesis and is famous

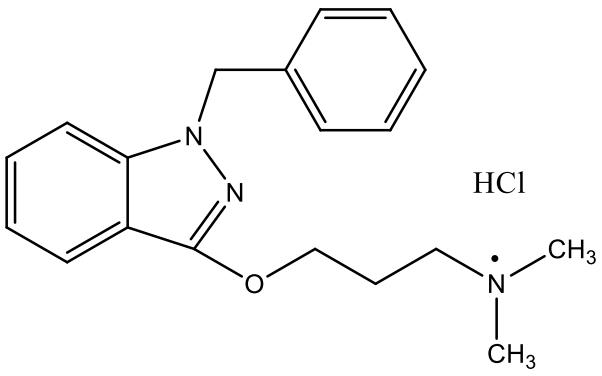
for its anti-platelet effect as well [92]. It is soluble in methanol, ethanol, dimethyl sulfoxide and acetone.



Aspirin

Benzydamine

Benzydamine, 3-(1-benzyl-1*H*-indazol-3-yloxy)-*N*,*N*-dimethylpropan-1-amine (structure given below), exists as the hydrochloride. This is a locally-acting NSAID with analgesic and anaesthetic characteristics for relieving pain. This is also used for the treatment of mouth and throat inflammation. This is soluble in water.



Benzydamine

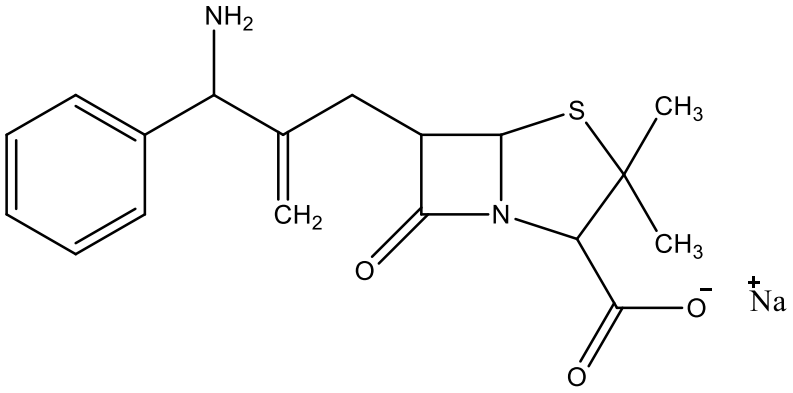
1.11.2. Antibiotics

Following antibiotic were selected for present studies.

Ampicillin sodium

Ampicillin, sodium 6-(2-(amino (phenyl) methyl) allyl)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylate (structure given below), is a β-lactam antibiotic and is member of aminopenicillin family. It is effective against Gram-

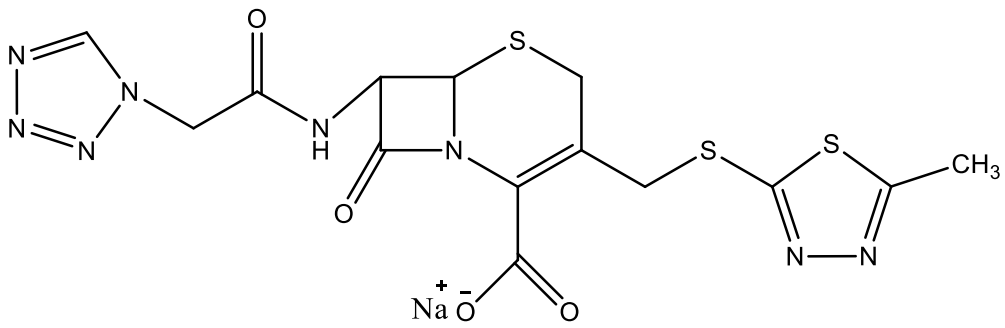
positive and many Gram-negative bacteria. Amino group of its structure makes it different from other antibiotics of the penicillin group. This amino group helps ampicillin to penetrate outer membrane of the gram-negative bacteria. It is soluble in water.



Ampicillin sodium

Cefazolin sodium

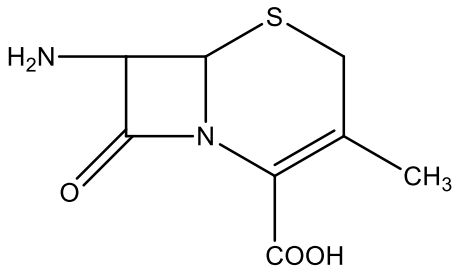
Cefazolin sodium, sodium 7-(2-(1H-tetrazol-1-yl)acetamido)-3-(((5-methyl-1,3,4-thiadiazol-2-yl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (structure given below), is a 1st generation cephalosporin antibiotic. It is commonly used to treat infections of the bone, joint, lung, heart valve, blood, urinary tract, stomach, and especially skin. It is soluble in water.



Cefazolin sodium

7-ADCA

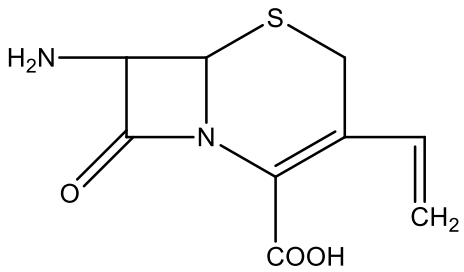
7-ADCA, 7-amino deacetoxycephalosporanic acid (structure given below), belongs to the β-Lactam group and it is one of the building blocks of cephalosporin antibiotics. It is precursor of cephalexin and ampicillin. It is soluble in dimethylformamide.



7-ADCA

7-AVCA

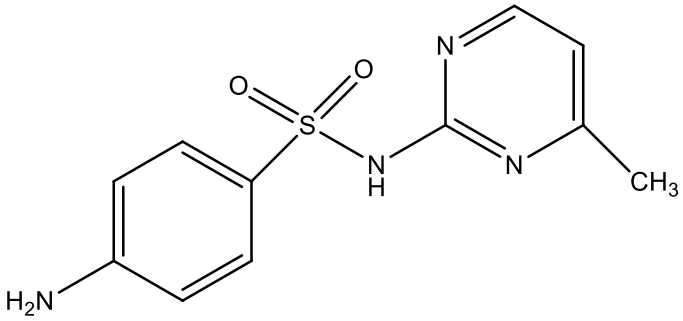
7-AVCA, 7-amino-3-vinylcephalosporanic acid (structure given below), is member of β-lactam group and it is a precursor of cefdinir and cefixime. It is soluble in dimethylformamide.



7-AVCA

Sulfamirazine

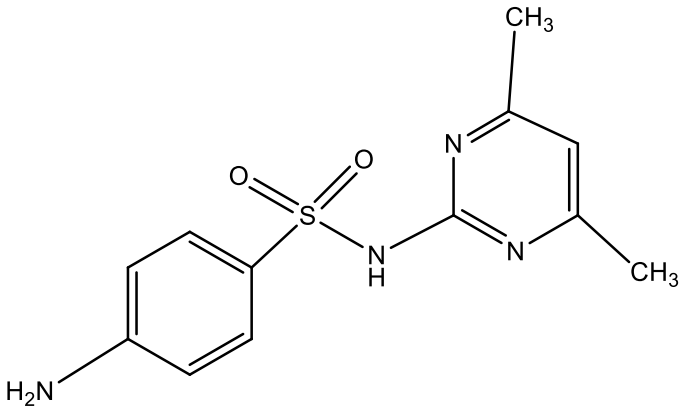
Sulfamerazine, 4-amino-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (structure given below), is used as an antibiotic. This antibiotic can be used to treat bronchitis, urinary tract infections and prostatitis. This is a sulfonamide drug and inhibits bacterial synthesis of dihydrofolate (an enzyme involved in folate synthesis in bacteria). It is soluble in dichloromethane and dimethyl sulfoxide.



Sulfamirazine

Sulfamethazine

Sulfamethazine, 4-(((4,6-dimethylpyrimidin-2-yl)methyl)sulfonyl)aniline (structure given below), belongs to sulfonamides. It is commonly used for the treatment of bronchitis, urinary tract infections and prostatitis. Mechanism of action is similar to sulfamerazine. It is soluble in dichloromethane.

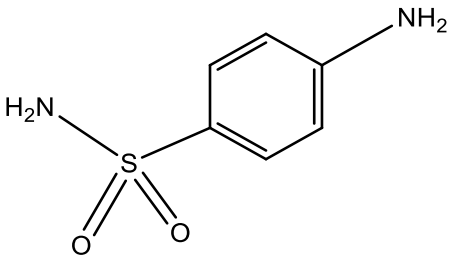


Sulfamethazine

Sulfanilamide

Sulfanilamide, 4-aminobenzenesulfonamide (structure given below), is an antibiotic with a molecule containing sulfonamide functional group. It can be used for the treatment of vulvovaginitis, an infection by *Candida albicans*. It inhibits an important bacterial enzyme dihydropteroate synthetase, necessary for the synthesis of folic acid,

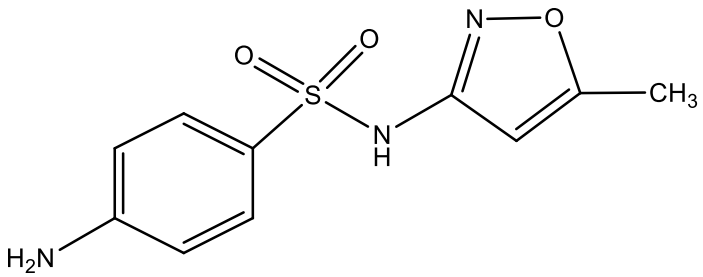
without it bacteria cannot survive. It is soluble in dichloromethane and dimethyl sulfoxide



Sulfanilamide

Sulfamethoxazole

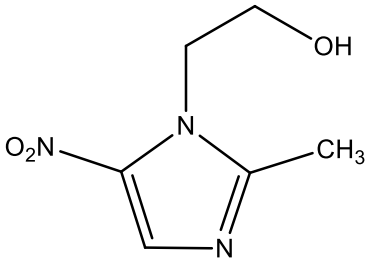
Sulfamethoxazole, 4-amino-N-(5-methylisoxazol-3-yl) benzenesulfonamide (structure given below), is a bacteriostatic antibacterial agent that inhibits folic acid synthesis in the susceptible bacteria. It can be used for the treatment of bacterial infections resulting in bronchitis, pneumonia, prostatitis, middle ear and urinary tract infections. It is soluble in methanol, ethanol and dichloromethane.



Sulfamethoxazole

Metronidazole

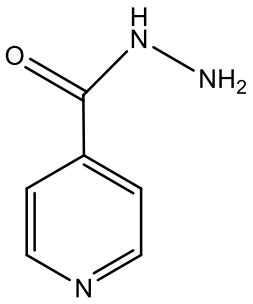
Metronidazole, 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethanol (structure given below), is a synthetic antibacterial as well as antiprotozoal agent of the nitroimidazole class. Metronidazole is exceptionally effective against many anaerobic bacterial infections. It is soluble in ethanol and dimethyl sulfoxide.



Metronidazole

Isoniazid

Isoniazid, isonicotinohydrazide (structure given below), is an antibacterial agent (nicotinamide derivative) that primarily acts as a tuberculostatic. It inhibits the synthesis of nucleic acids, lipids, and mycolic acid of the cell walls of bacteria belonging to genus Mycobacterium, especially *M. tuberculosis*. It is soluble in methanol, acetone and dimethyl sulfoxide.



Isoniazid

1.12. Biological evaluation of prodrugs

Drugs can be assessed on their biological activities. As antibiotics and anti-inflammatory drugs are the main focus of the present study so prodrugs synthesized from these drugs should be at least monitored for their parent basic properties. Biological properties can be studied in vitro, in vivo or through computational analysis. A key benefit of *in vitro* work is to permit a great level of convenience and simplification in the system under study. Computational studies can be used to point

toward “performed on the computer or via computer simulation”. Several software are available for this purpose [93].

1.12.1. Anti-bacterial study

There is usually no single complete bioassay available to evaluate the antimicrobial activity of a drug. Hence, the antibacterial evaluation process usually involves the use of more than one bioassay methods then careful assessment and comparison of all the observed data in order to obtain an appropriate conclusion [94]. Antimicrobial activity can be measured by following methods.

a) Agar dilution method [95]

b) Bio autographic method [96]

c) Agar diffusion method [97, 98]

In the present study, agar diffusion method was used in antibacterial activity while agar dilution method was used in anti-tuberculosis activity. In agar diffusion method, wells are usually cut in seeded agar and the sample is then introduced directly into these wells while in agar dilution method the medium is inoculated with the test organism and the samples to be tested are mixed with the inoculated medium.

1.12.2. Enzyme inhibition assay

Enzyme assay are the laboratory methods used for measuring enzymatic activity (enzyme inhibition and enzyme kinetics). Enzyme assays can be categorised as continuous assays and discontinuous assays. In present study, continuous spectrophotometric assay type was selected. In this type, course of the reaction was followed by measuring change in light absorption of the assay solution. In this study,

5-lipoxygenase (5-LOX), acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and α-chymotrypsin were the enzymes selected for the enzyme inhibition assays.

5-LOX is an enzyme that in human beings is encoded by ALOX5 gene. This enzyme transforms essential fatty acids into leukotrienes (eicosanoid inflammatory mediators produced in white blood cells). Leukotrienes are produced in leukocytes (white blood cells) by the oxidation of an essential fatty acid arachidonic acid by 5-LOX [99]. Inhibiting 5-LOX blocks the biosynthesis of harmful inflammatory leukotrienes [100].

Acetylcholinesterase is an enzyme that hydrolyses neurotransmitter acetylcholine while butyrylcholinesterase is a non-specific cholinesterase enzyme that hydrolyses many different choline esters. BChE is very similar to the neuronal AChE. The term "serum cholinesterase" is generally used for these two enzymes. Assay of butyrylcholinesterase activity in plasma can be used as a liver function test as both hypercholinesterasemia and hypocholinesterasemia indicate pathological processes in liver. Both of these enzymes play role in increasing Alzheimer’s disease. There are evidences that anti-inflammatory therapies proved helpful in slowing the onset of the Alzheimer's disease. Derivatives of NSAIDs have been used successfully in the search for multifunctional anti-Alzheimer's disease agents with good safety [101].

1.12.3. Toxicity of NSAIDs

All drugs are considered toxic at some level; the level of toxicity can be observed by drug’s effects on the target which may be an organism, organ, tissue or a cell. Toxicity can be acute, sub chronic or chronic in nature depending upon the exposure of the target to the drug. Major challenge in drug discovery is to find a margin between efficacy and toxicities, enough to provide clinical benefits to patients while

avoiding to put them at unnecessary risk of side effects. Drugs like NSAIDs and antibiotics may cause gastrointestinal and hepatic toxicity [102,103].

The most pronounced and fatal adverse effect of NSAIDs is on gastrointestinal tract (GIT). The GIT toxic effects include dyspepsia, ulcers and bleeding [104]**.** Around 30 million people world over consume these drugs on daily basis [105] and about 30% of these users may develop GIT toxicity. It has also been estimated that about one third of the arthritis patients are victims of these adverse effects. A careful survey indicated that approximately 0.1 million patients are hospitalized annually for NSAID-related GIT complications. This indicates that the deaths exceed than those by AIDS and cervical cancer [106]. Apparently these toxic effects are due to presence of carboxylic acid moiety in most of the NSAIDs. This alarming situation warrants structural modification(s) in existing NSAIDs where these effects can be avoided by sequestering the carboxylic group. In order to achieve this, different strategies have been adopted in modifying the existing NSAIDs [107**]** or synthesizing new molecules [108]. One of the objectives of the present work was to derivatize some of the NSAIDs with other drug molecules, having amino group in them, through amide formation. This type of derivatization would afford prodrugs with dual effect with elimination of toxic effects due to free carboxylic groups in NSAIDs.

Mutual prodrugs can be used to decrease toxic and adverse effects while increasing the pharmacological effects.

2. Experimental

2.1.Materials

The drugs used were: cefazolin Sodium (GlaxoSmithKline, Karachi), ampicillin (Sigma-Aldrich, Germany), sulfamethoxazole (Sigma-Aldrich, Germany), sulfamethazine (Sigma-Aldrich, Germany), sulfamerazine (Sigma-Aldrich, Germany), sulfanilamide (Sigma-Aldrich, Germany), isoniazid (Sigma-Aldrich, Germany), 7-aminodesacetoxycephalosporanic acid (7-ADCA) (Pharmagen, Lahore), 7-amino-3-vinylcephalosporanic acid (7-AVCA) (Pharmagen, Lahore), metronidazole (Nawabsons, Lahore), ibuprofen (Nawabsons, Lahore), flurbiprofen (Nawabsons, Lahore), benzydamine hydrochloride (Adamjee, Karachi), and aspirin (Sigma-Aldrich, Germany).

Dichloromethane, N, N´-dimethylformamide (DMF), triethyl amine, 4-dimethylaminopyridine (DMAP), N, N'-dicyclohexylcarbodiimide (DCCI), acetonitrile, petroleum ether, methanol, diethyl ether, oxalyl chloride were of analytical grade from E. Merck, Germany.

All other chemicals used in the experiments were of analytical grade and were used without further purification or specified otherwise. The solvents used were purified by distillation and dried before use. Glassware used for the reactions was oven dried. Round bottom used were fitted with rubber septa. Reactions were conducted under a positive pressure of nitrogen. Cannulae were used to transfer moisture sensitive liquids.

2.2. Chromatography

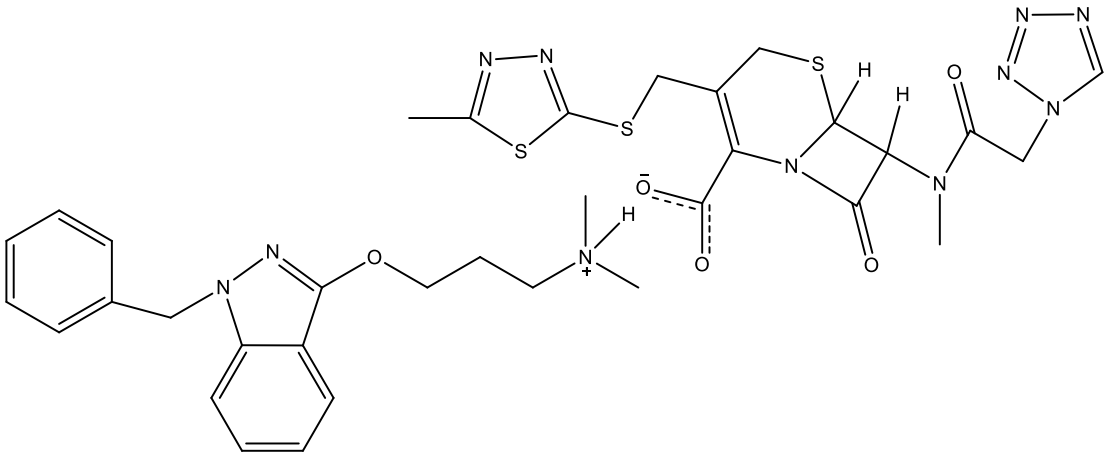
Flash chromatography was performed using silica gel grade 9385 (Si, 230-400 mesh, pore size 60 Å, Sigma Aldrich) under nitrogen. Column taken was 46 cm long with 30 mm diameter. Purity of the prodrugs was monitored on pre-coated silica gel GF-254 (dimensions 20×20 cm 0.5 mm thick) (Merck) thin layer chromatography plates. TLC plates were visualised by exposure to UV light. While performing these chromatographic procedures, different solvent systems of low, medium and high polarity were used according to the requirement.

2.3. Synthesis

Three methods were used for synthesis of mutual prodrugs of antibiotics and NSAIDs. In the first method, the antibiotics and NSAIDs were mixed together in an appropriate solvent in equvimolar quantities and stirred at ambient temperature or refluxed. By this method only the reaction between cefazolin sodium and benzydamine hydrochloride was successful. In the second method, the NSAIDs containing carboxylic groups were converted to their acid chlorides by use of thionyl chloride or oxalyl chloride. The acid chlorides thus obtained were allowed to react with the antibiotics containing amino or hydroxyl groups. With the use of thionyl chloride, aspirin could be coupled with sulfamethoxazole, sulfamethazine and sulfanilamide. With the use of oxalyl chloride the acid chlorides of ibuprofen and flurbiprofen could be coupled with sulfamethazine, sulfamerazine, sulfamethoxazole, sulfanilamide, metronidazole, isoniazid, 7-ADCA and 7-AVCA. In the third method, NSAIDs and antibiotics were allowed to react with each other in the presence the coupling agent, DCCI, and the catalyst, DMAP. By this method ampicillin could be coupled with ibuprofen and flurbiprofen.

2.3.1. Cefazolin sodium-benzydamine hydrochloride HCl mutual prodrug, 3-[(1-benzyl-1H-indazol-3-yl)-oxy]-N,N-dimethyl-propan-1-aminium3-{[(5-methyl-1,3,4-thia-diazol-2-yl)sulfan -yl] meth-yl}-8-oxo-7-[(1H-tetra-zol-1-yl)acetamido]-5-thia-1-aza-bicyclo-[4.2.0]octane-2-carboxylate (benzydaminium cephazolinate) (A)

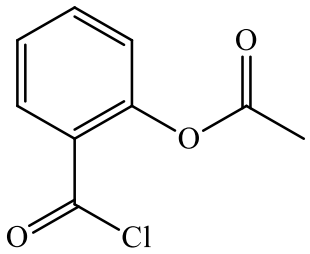
Cefazolin and benzydamine HCl were coupled according to a reported method [109] Cefazolin (47.6 mg, 10 mmol) and benzydamine hydrochloride (34.5 mg, 10 mmol) were dissolved in distilled water (10 mL) separately and mixed together and stirred briefly. The mixture was left at room temperature for 24 h. The product crystallized on standing, which was isolated by filtration and dried in air at room temperature. The product produced a single spot on thin layer chromatographic plate (pre-coated silica gel GF-254 0.5 mm thick, Merck; 1:1 water-methanol); CHNS calculated ($C_{34}H_{39}N_{11}OS_3$): C, 52.49; H, 5.05; N, 19.81; O, 10.28; S, 12.37; found: C, 52.51; H, 4.92; N, 19.79; O, 10.29; S, 12.31



(A)

2.3.2. Aspirin chloride, 2-(chlorocarbonyl) phenyl acetate [110]

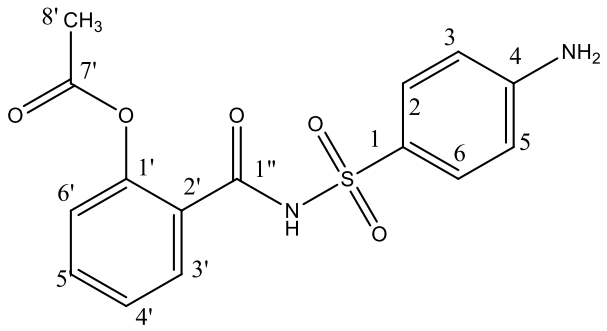
Aspirin (180.0 mg, 1.0 mmol) was dissolved in toluene (20 mL) under nitrogen cover. To this aspirin solution, thionyl chloride (l mL) and catalytic amount (0.01 mL) of N, N´-dimethylformamide was added. The mixture was heated under nitrogen cover at 70 ^{o}C for 1 h (the completion of reaction was monitored by TLC (pre-coated silica gel GF-254 0.5 mm thick, Merck; 1:2chloroform: ethyl acetate). The solvent was removed under reduced pressure to obtain aspirin chloride as white crystals. ^{1}H-NMR: 7.45-7.82 (m, 4H,H-3′, H-4′, H-5′,H-6′), 2.28 (s, 3H, CH_3-8′)**;** ^{13}C-NMR: 169.0 (C-7′), 167.9 (C-1), 155.1 (C-1′), 135.7 (C- 5′), 131.6 (C-3′), 128.1 (C-2′), 125.8(C-4′), 120.3 (C-6′), 20.3 (C-8′).



2.3.3. Aspirin-sulfanilamide mutual prodrug, 2-(((4-aminophenyl) sulfonyl) carbamoyl) phenyl acetate (A20)

The aspirin chloride was coupled with sulfanilamide according to a reported method [111]. To a solution of sulfanilamide (138 mg, 0.80 mmol) in dry dichloromethane (15 mL) triethyl amine (0.3 mL) was added under inert atmosphere. This was followed by addition of aspirin chloride (159 mg, 0.80 mmol) and DMAP (12 mg, 0.098 mmol) in dry dichloromethane (15 mL). The reaction mixture was stirred overnight. After this time the reaction was found to be complete as evidenced by no further change in TLC (pre-coated silica gel GF-254, Merck; 1:1 ethyl acetate:

petroleum ether) pattern. The solvent was evaporated to about one fourth of the volume by rotary evaporator. A white powder was isolated by filtration. The product was purified by column chromatography (Silca gel, 1:1 ethyl acetate: petroleum ether). The product (A20) was dried under reduced pressure. Yield: 153 mg (66.5%) ; λ_{max}: 270 nm; IR (KBr, cm^{-1}): 3473 (Ar–NH), 3230 ν(–NHCO), 1697 (–C=O amide), 1597 (–NH), 1330 (–CN), 1240 (–COC), 1147 (–S=O), 839 (Ar); 1H-NMR $CDCl_3$(δ); 7.8 (br, s., 1H, –CONH), 7.58 (dd, J=8, 2.4 Hz, 2H, H-2), 7.3-7.6 (m, 4H, H-3′, H-4′, H-5′, H-6′), 6.65 (dd, J=8.1, 2.9 Hz, 2H, H-3, H-5), 5.4 (s, 2H), 2.0 (s, 3H, CH_3); ^{13}C-NMR (δ): 173.1(C-7′), 160.8 (C-1′′), 154.0 (C-4), 152.3 (C-1′), 133.3 (C-5′), 130.76 (C-1), 128.7 (C-2), 124.5 (C-3′), 115.1 (C-2′), 113.38 (C-6′), 112.9 (C-3), 19.9 (C-8′); ESI-MS: 333.0551, [M-H^+] 334.0547; $C_{15}H_{14}N_2O_5S$.

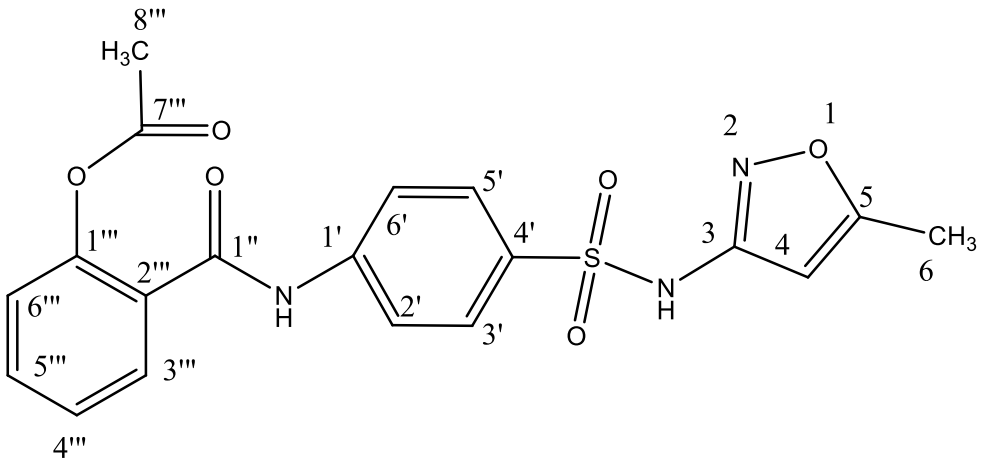


(A20)

2.3.4. Synthesis of aspirin –sulfamethoxazole mutual prodrug, 2-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) carbamoyl) phenyl acetate (A24)

This compound was synthesized as per procedure for A20 above by replacing sulfanilamide with sulfamethoxazole (202 mg, 0.80 mmol). The purified product (A24) was then characterized. Yield:185mg (64%) ; λ max : 278 nm; 1H-NMR $CDCl_3$ (δ); 7.8 (br. S., 1H, –CONH), 7.46 (dd, J=7.8, 2.2 Hz, 2H, H-3′, H-5′), 7.43 (dd, 7.8,

2.1 Hz, 2H, H-2′, H-6′), 7.20-7.40 (m, 4H, H-3‴, H-4‴, H-5‴, H-6‴), 6.7 (s, 1H, NH), 6.2 (s, 1H, H-4), 2.2 (s, 3H, H-6), 2.0 (s, 3H, H-8‴); 13C-NMR (δ): ;176.7 (C-3), 173.4 (C-7‴), 171.1 (C-5), 160.9 (C-1″), 150.8 (C-1‴), 140.9 (C-1′), 133 (C-5‴), 128.1 (C-3′), 127.7 (C-4‴), 123.4 (C-3‴), 115.1 (C-2‴), 114.9 (C-6‴), 106.5 (C-2′), 90.0 (C-4), 18.2 (C-8‴), 12.4 (C-6); ESI-MS: 416.0736 [M+H+], 415.0739; $C_{19}H_{17}N_3O_6S$.

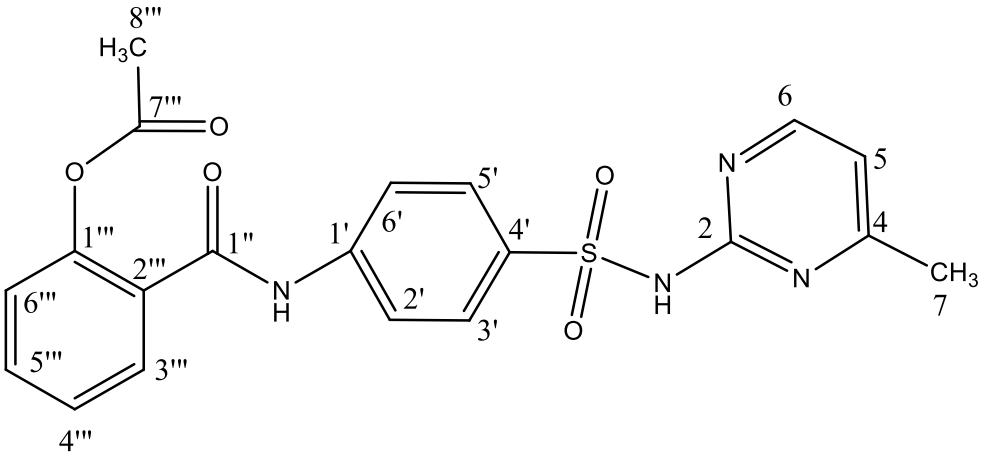


(A24)

2.3.5. Synthesis of aspirin-sulfamerazine mutual prodrug, 2-((4-(N-(4-methylpyrimidin-2-yl)sulfamoyl) phenyl) carbamoyl) phenyl acetate (A25).

This compound was synthesized as per procedure for **A20** above by replacing sulfanilamide with sulfamerazine (211 mg, 0.80 mmol). The purified product **(A25)** was then characterized. Yield: 203mg (68.35%) ; λ_{max} : 278 nm; FT-IR (KBr) ν cm^{-1}: 3182 ν(–NHCO), 1683 (–C=O amide), 1589 (–NH), 1323 (–CN), 1153 (–S=O), 752 (Ar), 698 (pyrimidine); ^{1}H-NMR, $CDCl_3$, TMS reference, MHz: (δ); 8.2 (s, 1H, –CONH), 8.1 (s, 1H, –CONH), 7.65 (dd, J=8.1, 2.4 Hz, 2H, H-2′, H-6′), 7.33 (dd, J=8.1, 2.1 Hz, 2H, H-3′), 7.25-7.55 (m, 4H, H-3‴, H-4‴, H5‴, H-6‴), 6.75 (d, J=7.9

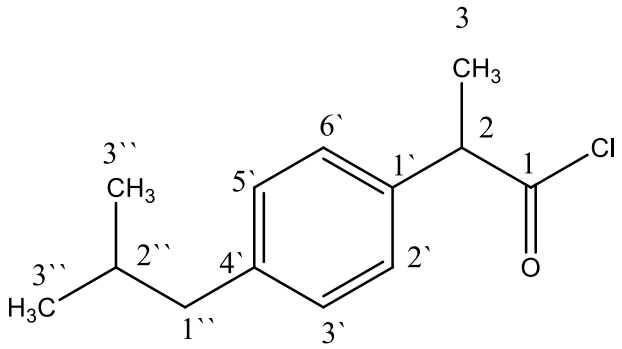
Hz, 1H, H-5), 5.16 (d, J=7.9 Hz, 1H, H-6), 2.38 (s, 3H, H-7), 2.2 (s, 3H, H-8‴); ^{13}C-NMR (ppm): 173.4 (C-7‴), 160.96 (C-1″), 158.4 (C-6), 150.8 (C-2), 140.9 (C-1′), 140.8 (C-4′), 133.0 (C-5‴), 128.1 (C-5′), 127.8 (C-4‴), 123.4 (C-3‴), 115.1 (C-2‴), 114.9 (C-5), 106.5 (C-2′), 18.22 (C-7), 14.6 (C-8‴); ESI-MS: 427.0896 [M+H$^+$], 426.0897; $C_{20}H_{18}N_4O_5S$.



(A25)

2.3.6. Synthesis of ibuprofen chloride, 2-(4-isobutylphenyl) propanoyl chloride [112]

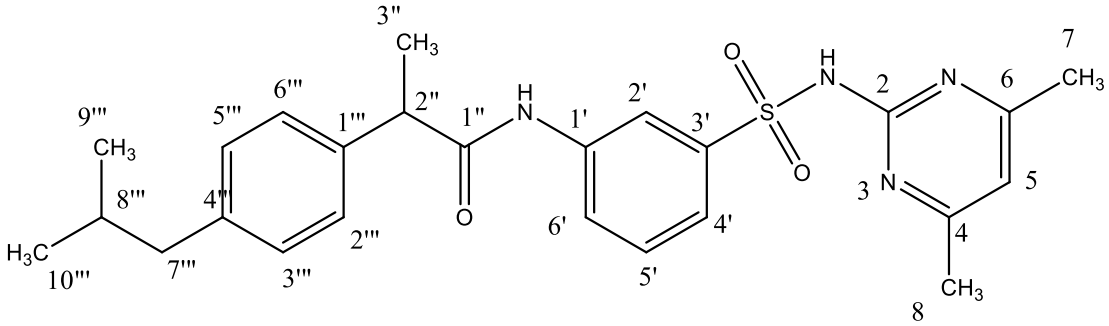
Ibuprofen (1.5000 g, 7.28 mmol) was dissolved in dry dichloromethane (30 mL). To this dry DMF (0.03 mL) was added as catalyst. This was followed by drop wise addition of oxalyl chloride (3 mL) as the chlorinating agent; during the addition temperature was maintained at 0-5 ºC. The resulting mixture was stirred for 12 h at about 25ºC. After this the solvent was evaporated on a rotary evaporator. An oily product was obtained. ^{1}H-NMR $CDCl_3$(δ); 7.24 (dd, J=7.2, 1.9 Hz, 2H, H-2′, H-6′), 7.14 (dd, J=7.3, 2.0 Hz, 2H, H-3′, H-5′), 3.81 (q, J=7.4 Hz, 1H, H-2), 2.48 (d, J=7.4 Hz, 2H, H-7′), 1.87 (m, 1H, H-8′), 1.54 (d, J=7.0 Hz, 3H, H-3), 0.91 (d, J=6.7 Hz, 6H, H-3``); ^{13}C-NMR: 173.6 (C-1), 140.3 (C-4′), 133.1 (C-1′), 129.0 (C-5′), 128.7 (C-6′), 55.7 (C-2), 44.5 (C-7′), 29.0 (C-8′), 22.8 (C-9′), 16.4 (C-3).



Ibuprofen chloride

2.3.7. Synthesis of ibuprofen-sulfamethazine mutual prodrug, N-(3-(N-(4, 6-dimethylpyrimidin-2-yl) sulfamoyl) phenyl)-2-(4-isobutyl phenyl) propanamide (A2).

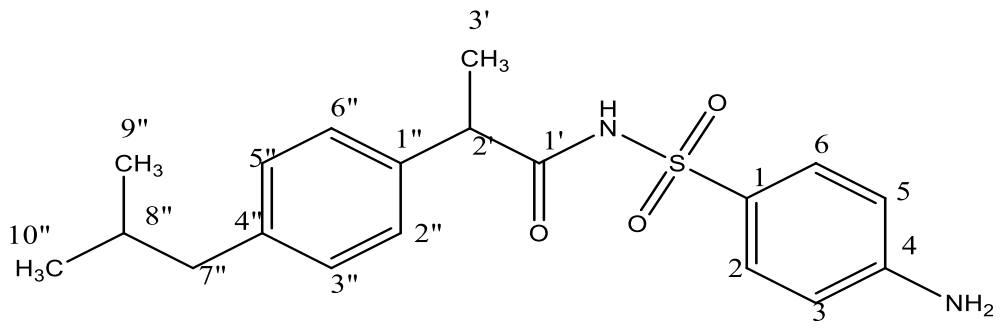
This compound was synthesized as per procedure for A20 above by replacing sulfanilamide with sulfamethazine (222.4 mg, 0.80 mmol) and aspirin chloride by ibuprofen chloride (180 mg, 0.80 mmol). The purified product (A2) was then characterized. Yield: 243.4 mg (64.9%); λ_{max} = 290 nm; IR (KBr) ν cm^{-1}: 3334 ν (–NH), 1708 (–C=O), 1583 (–NH), 1519 (–CH), 1153 (–CN); ^{1}H-NMR $CDCl_3$(δ); 8.2 (s, 1H, –CONH), 7.77 (m, 1H, H-4′), 7.7 (m, 1H, H-5′), 7.53, (s, 1H, H-5), 7.30 (d, J=2.1Hz, 1H, H-2′), 7.19 (dd, J=7.9, 2.5 Hz, 2H, H-2‴), 7.10 (m, 1H, H-6′), 7.06 (dd, J=7.9, 2.2 Hz, 2H, H-3‴, H-5‴), 4.2 (s, 1H, CONH), 3.65 (q, J=7.0Hz, 1H, H-2″), 2.25(s, 6H, H-7, H-8), 2.40 (d, J=6.8Hz, 2H, H-7‴), 1.8 (m, 1H, H-8‴), 1.5 (d, J=7.0 Hz, 3H, H-3″), 0.85 (d, J=6.6 Hz, 6H, H-9‴, H-10‴); ^{13}C-NMR (δ): 172.2 (C-1″), 169.5 (C-4), 169.1 (C-6), 153.2 (C-2), 144.6 (C-1′), 139.4 (C-1‴), 135.5 (C-3′), 131.2 (C-5′), 130.9 (C-4‴), 129.1 (C-3‴), 125.5 (C-6‴), 123.3 (C-4′), 118.1 (C-6′), 115.5 (C-2′), 107.9 (C-5), 52.1 (C-2″), 44.4 (C-7‴), 30.4 (C-8‴), 22.1 (C-9‴, C-10‴), 18.6 (C-3″); ESI-MS: 467.2111 [$M+H^+$]; 466.2110; $C_{25}H_{30}N_4O_3S$.



(A2)

2.3.8. Synthesis of ibuprofen-sulfanilamide mutual prodrug, N-((4-aminophenyl) sulfonyl) -2- (4-isobutylphenyl) propanamide (A3)

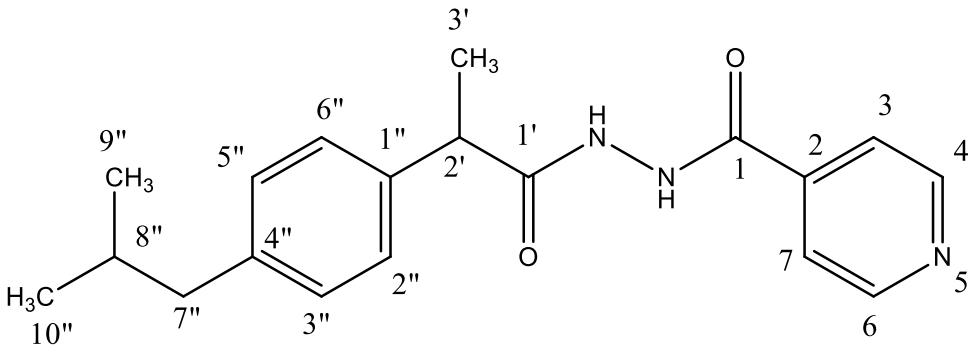
This compound was synthesized as per procedure for A20 above by replacing aspirin chloride with ibuprofen chloride (180 mg, 0.80 mmol). The purified product (A3) was then characterized. Yield: 206 mg (71%) ; IR (KBr) ν cm^{-1}: 3475 (–NH), 3329 (amide), 1720 (–C=O), 1602 (–C=C), 1484 (–CH), 850 (Ar); ^{1}H-NMR $CDCl_3$(δ); 7.45 (dd, J=7.9, 2.1 Hz, 2H, H-2), 7.19 (dd, J=7.9, 2.5 Hz, 2H, H-2″, H-6″), 7.06 (dd, J=7.9, 2.2 Hz, 2H, H-3″, H-5″), 6.95 (dd, J=7.9, 2.0 Hz, 2H, H-3, H-5), 5.55 (s, 1H, –CONH), 5.5 (s, 2H, $–NH_2$), 3.52 (q, J=7.1 Hz, 1H, H-2′), 2.50 (d J=6.8 Hz, 2H, H-7″), 1.89 (m, 1H, H-8″), 1.28 (d, 3H, H-3′), 0.88 (d, J=6.6 Hz, 6H, H-9″, H-10″); ^{13}C-NMR (δ): 173 (C-1′), 153.9 (C-4), 135.4 (C-1), 130.9 (C-4″), 128.4 (C-6″), 127.1 (C-5″), 123.5 (C-6), 115.2 (C-5), 49.0 (C-2′), 44.86 (C-7″), 30.66 (C-8″), 20.0 (C-3′), 24.63 (C-9″,C-10″); ESI-MS: 361.15 [$M+H^+$]; 360.15 $C_{19}H_{24}N_2O_3S$.



(A3)

2.3.9. Synthesis of prodrug of ibuprofen and isoniazid, N'-(2-(4-isobutylphenyl) propanoyl) isonicotinohydrazide (A4)

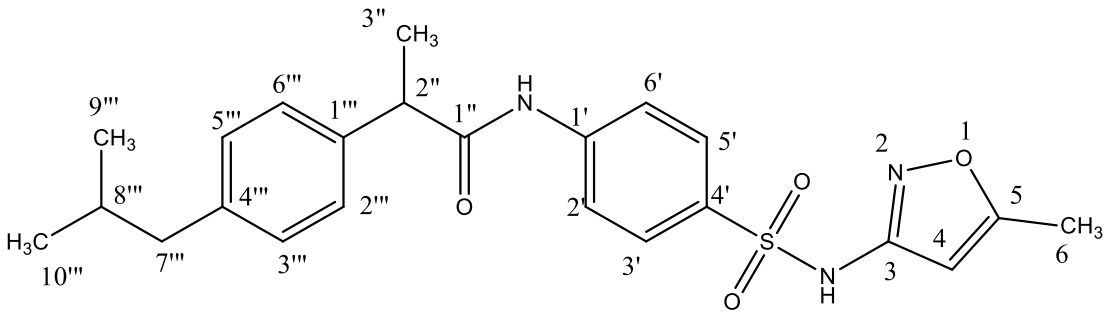
This compound was synthesized as per procedure for A20 above by replacing aspirin chloride with ibuprofen chloride (180 mg, 0.80 mmol) and sulfanilamide by isoniazid (110 mg, 0.80 mmol). The purified product (A4) was then characterized. Yield: 178 mg (68.15). Product (A4) was obtained and characterized; UV-VIS λ_{max} 225 nm ; IR (KBr) ν cm^{-1}: 3337 (–NHC=O), 1723 (–C=O), 1595 (–NH), 1452 (–CH), 836 (–Ar); ^{1}H-NMR $CDCl_3$(δ); 8.75 (dd, J=8.3, 2.0 Hz, 2H, H-4 H-6), 8.2 (s, 2H, –CONH), 7.6 (dd, J=8.3, 2.5 Hz, 2H, H-3, H-7), 7.15 (dd, J=8.1, 2.2 Hz, 2H, H-2″, H-6″), 7.08 (dd, J=8.1, 2.2 Hz, 2H, H-3″, H-5″), 3.7 (q, J=7.2 Hz, 1H, H-2′), 2.49 (d, J=6.8 Hz, 2H, H-7″), 1.88 (m, 1H, H-8″), 1.5 (d, J=6.8 Hz, 3H, H-3′), 0.85 (d, J=6.5 Hz, 6H, H-9″, H-10″); ^{13}C-NMR (δ): 168.8 (C-1), 148.9 (C-4), 143.5 (C-2), 142.9 (C-1″), 130.9 (C-4″), 128.4 (C-2″), 127.1 (C-5″), 122.1 (C-3), 53.4 (C-2′), 44.8 (C-7″), 30.6 (C-8″), 24.9 (C-3′), 24.6 (C-9″, C-10′); ESI-MS: 326.1863 [$M+H^+$], 325.1851; $C_{19}H_{23}N_3O_2$.



(A4)

2.3.10. Synthesis of prodrug of ibuprofen and sulfamethoxazole, 2-(4-isobutylphenyl)-N-(4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) propanamide (A5)

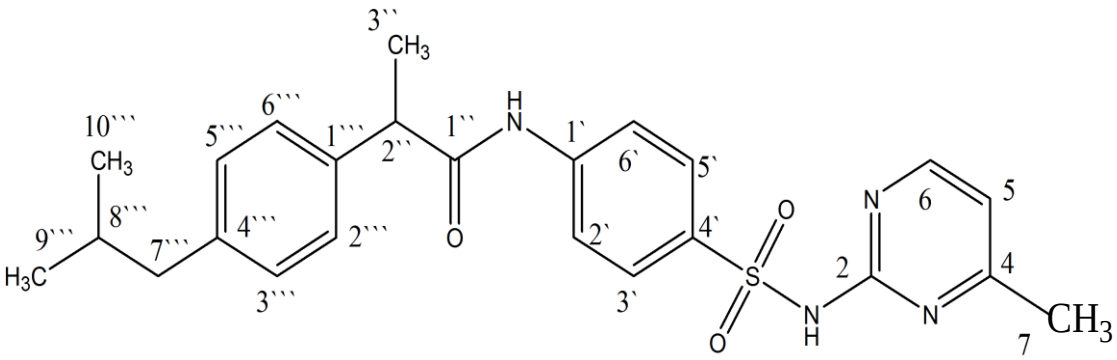
This compound was synthesized as per procedure for A20 above by replacing sulfanilamide with sulfamethoxazole (202.4 mg, 0.80 mmol) and aspirin chloride by ibuprofen chloride (180 mg, 0.80 mmol). The purified product (A5) was then characterized. Yield: 251.1 mg 70.8%); λ_{max} = 260 nm; IR (KBr) ν cm^{-1} 3219 (–NHC=O), 2953 (=CH), 1695 (–C=O), 1595 (–C=C– Ar), 1325 (–CN), 1161 (–CO); ^{1}H-NMR $CDCl_3$(δ); 9.6 (s, 1H, –SNH), 7.9 (dd, J=7.8, 2.2 Hz, 2H, H-3′), 7.45 (dd, J=7.8, 2.1 Hz, 2H, H-2′, H-6′), 7.14 (dd, J=7.9, 2.0 Hz, 2H, H-3‴, H-5‴), 7.09 (dd, J=7.9, 1.9 Hz, 2H, H-2‴, H-6‴), 6.0 (s, 1H, –CONH), 5.9 (s, 1H, H-4), 3.5 (q, J=7.1 Hz, 1H, H-2″), 2.3 (d, J=6.9 Hz, 2H, H-7‴), 1.9 (s, 3H, H-6), 1.7 (m, 1H, H-8‴), 1.3 (d, J=7.0 Hz, 3H, H-3″), 0.89 (d, J=6.5 Hz, 6H, H-9‴, H-10‴); ^{13}C-NMR (δ): 180.2 (C-2), 174.4 (C-5), 169.4 (C-1″), 142.7 (C-1′), 141.1 (C-4′), 140.8 (C-1‴), 129.3 (C-4‴), 127.9 (C-3‴), 127.8 (C-3′), 127.3 (C-6‴), 114.2 (C-6′), 96.2 (C-4), 47.5 (C-2″), 45.4 (C-7‴), 30.5 (C-8‴), 22.7 (C-9‴, C-10‴), 19.1 (C-3″), 13 (C-6); ESI-MS: 442.17 [M+H^+], 441.17; $C_{23}H_{27}N_3O_4S$.



(A5)

2.3.11. Synthesis of ibuprofen and sulfamerazine, 2-(4-isobutylphenyl)-N-(4-(N-(4-methylpyrimidin-2-yl) sulfamoyl) phenyl) propanamide" (A10)

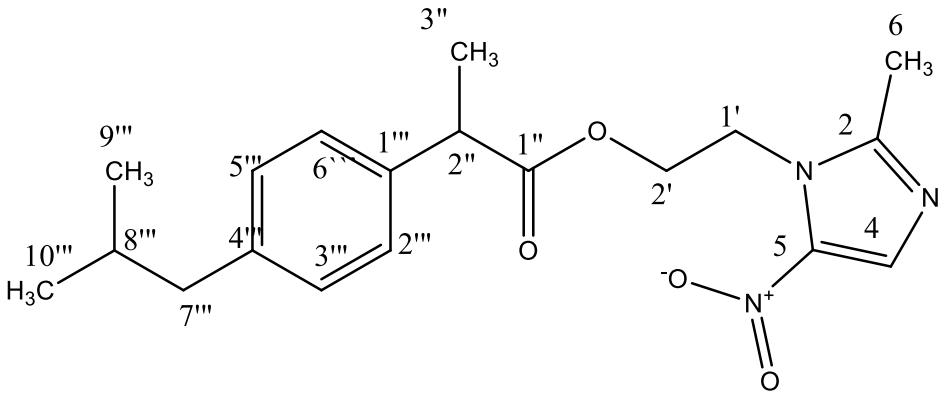
This compound was synthesized as per procedure for A20 above by replacing sulfanilamide with sulfamerazine (132 mg, 0.50 mmol) and aspirin chloride by ibuprofen chloride (180 mg, 0.80 mmol). The purified product (A10) was then characterized. Yield: 207 mg (63%) ; λ_{max} =235 nm; IR (KBr) ν cm^{-1}: 3205 ν(–NHCO), 2877(–CH), 1697 (–C=O), 1593 (–C=N), 1541 (–NH), 1352 (–CH), 1180 (–S=O), 866 (Ar); ^{1}H-NMR $CDCl_3$(δ); 8.2(s, 1H, –CONH), 8.14 (d, J=8.1 Hz, 1H, H-6), 7.25 (s, 1H, –SNH), 7.51 (dd, J=8.2, 2.5 Hz, 2H, H-2′, H-6′), 7.32 (dd, J=8.2, 2.2 Hz, 2H, H-3′, H-5′), 7.19 (dd, J=8.1, 2.0 Hz, 2H, H-2‴, H-6‴), 7.09 (dd, J=8.1, 2.2 Hz,2H, H-3‴, H-5‴), 6.55 (d, J=8.1 Hz, 1H, H-5), 3.81 (q, J=7.2 Hz, 1H, H-2″), 2.55 (s,3H, H-7), 2.353 (d, J=6.8 Hz, 2H, H-7‴), 1.91 (m, 1H, H-8‴), 1.5 (d, J=6.8 Hz, 3H, h-3″), 0.89 (d, J=6.5 Hz, 6H, H-9‴, H-10‴); ^{13}C-NMR (δ): 172.9 (C-1″), 156.8 (C-4), 156.5 (C-6), 168.93 (C-2), 151.58 (C-4′), 142.8 (C-1′), 135.7 (C-1‴), 130.4 (C-4‴), 128.9 (C-3′), 128.2 (C-3‴), 124.0 (C-2‴), 115.3 (C-5), 113.7 (C-2′), 47.77(C-2″), 47.7 (C-7‴), 31.2 (C-8‴), 24.07 (C-9‴, C-10‴), 24.0 (C-7), 19.0 (C-3″); ESI-MS: 453.1955[M+H^+], 452.1961; $C_{24}H_{28}N_4O_3S$.



(A10)

2.3.12. Synthesis of prodrug of ibuprofen and metronidazole 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethyl 2-(4-isobutylphenyl) propanoate (A11)

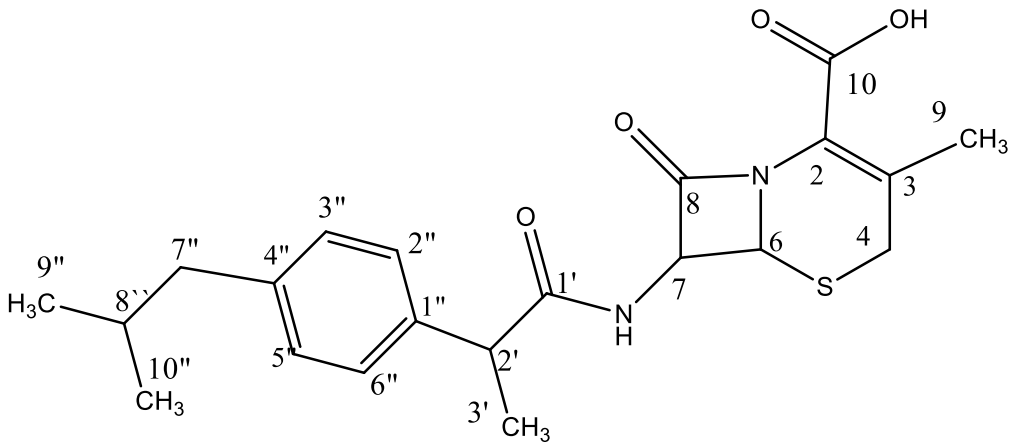
This compound was synthesized as per procedure for A4 above by replacing isoniazid with metronidazole (115 mg, 0.67 mmol). The purified product (A11) was then characterized. Yield: 194 mg (67%) ; IR (KBr) ν cm^{-1}: 2862 (–CH_3), 1734 (–C=O ester), 1641(–C=N), 1535 (–NO_2), 1465 (–CH_2), 1269 (–COC), 1074(–CO), 821 (Ar); ^{1}H-NMR $CDCl_3$(δ); 7.98 (s, 1H, H-4), 7.15 (dd, J=8.1, 2.2 Hz, 2H, H-3‴,H-6‴), 7.08 (dd, J=8.1, 2.0 Hz, 2H, H-3‴, H-5‴), 4.32 (t, J=5.9 Hz, 2H, H-2′), 4.01 (t, J=5.9 Hz, 2H, H-1′), 3.68 (q, J=7.2 Hz, 1H, H-2″), 2.32 (d, J=6.8, 2H, H-7‴), 2.46 (s, 3H, H-6), 1.75 (m, 1H, H-8‴), 1.25 (d, J=6.8 Hz, 3H, H-3″), 0.88 (d, J=6.5 Hz, 6H, H-9‴, H-10‴); ^{13}C-NMR (δ): 174.5 (C-1″), 152.8 (C-2), 140.8 (C-5), 138.1 (C-4), 135.7 (C-1‴), 130.4 (C-4‴), 128.2 (C-3‴), 124.0 (C-2‴), 63.4 (C-2′), 45.49 (C-2″), 45.49 (C-7‴), 19.17 (C-1′), 30.43 (C-8‴), 24.0 (C-9‴, C-10‴), 15.0 (C-3″), 14.49 (C-6); ESI-MS: 360.1918[M+H^+], 359.1927; $C_{19}H_{25}N_3O_4$.



(A11)

2.3.13. Synthesis of prodrug of Ibuprofen and 7-ADCA, 7-(2-(4-isobutyl phenyl)propanamido)-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid (A12).

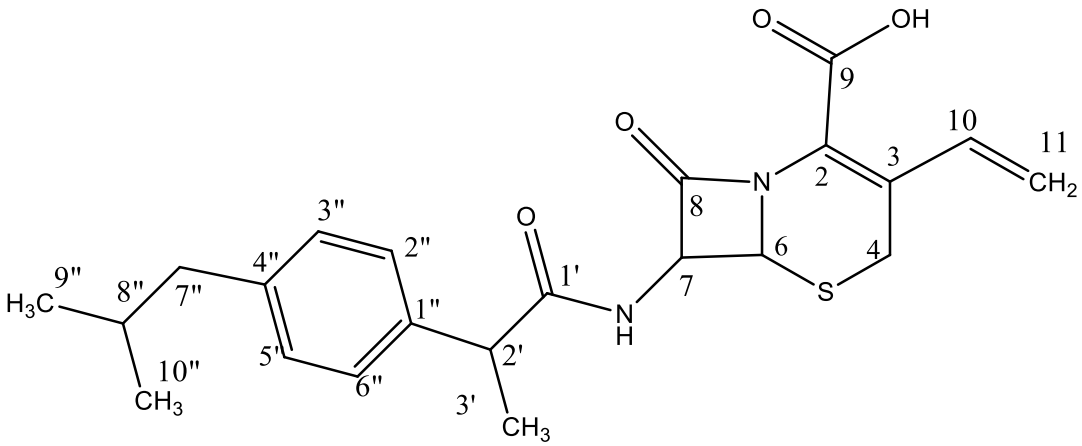
This compound was synthesized as per procedure for A4 above by replacing isoniazid with 7-ADCA (150 mg, 0.70 mmol). The purified product (A12) was then characterized. Yield: 213.4 mg (66%) ; λ_{max} 275 nm; IR (KBr) ν cm^{-1}: 3174 ν(–NHCO), 2941 (–CH_3), 1707 (–COOH), 1568 (–NH), 1463 (–CH_2), 1035 (–CN), 844 (Ar); ^{1}H-NMR $CDCl_3$(δ); 8.2 (s, 1H, –CONH), 7.15 (dd, J=8.1, 2.0,2H, H-2″), 7.08 (dd, J=8.1, 2.2, 2H, H-3″, H-5″), 5.6 (m, 1H, H-7), 5.2 (d, J=5.1 Hz, 1H, H-6), 3.82 (q, J=7.2, 1H, H-2′), 2.88 (s, 2H, H-4), 2.49 (d, J=6.8 Hz, 2H, H-7″), 1.8 (s, 3H, H-9), 1.7 (m, 1H, H-8″), 1.35 (d, J=6.8Hz, 3H, H-3′), 0.8 (d, J=6.5 Hz, 6H, H-9″, H-10″); ^{13}C-NMR (δ): 179.0 (C-10), 174.5 (C-8), 171.2 (C-1′), 142.9 (C-1″), 130.9 (C-4″), 128.9 (C-2), 128.4 (C-6″), 127.1 (C-3″), 125.1 (C-3), 52.58 (C-7), 46.41 (C-6), 46.11 (C-2′), 45.8 (C-7″), 39.49 (C-4), 30.16 (C-8″), 22.26 (C-9″, C-10″), 18.88 (C-3′), 8.43 (C-9); ESI-MS: 425.1511[M+Na^+], 402.1519; $C_{21}H_{26}N_2O_4S$.



(A12)

2.3.14. Synthesis of prodrug of ibuprofen and 7-AVCA, 7-(2-(4-isobutyl phenyl) propanamido-8-oxo-3-vinyl-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (A18)

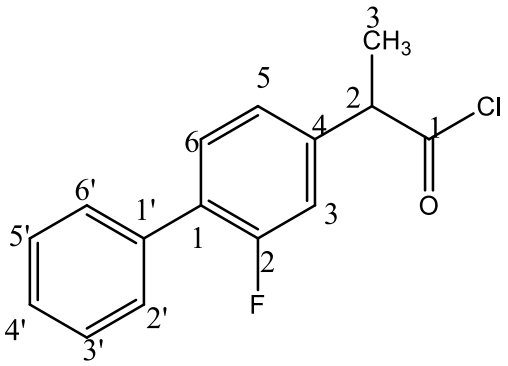
This compound was synthesized as per procedure for A4 above by replacing isoniazid with 7-AVCA (158 mg, 0.70 mmol). The purified product (A18) was then characterized. Yield: 219 mg (65.8%) ; UV-Vis. λ max 225 nm ; IR (KBr) ν cm^{-1}: 3450(OH carboxylic group), 3270 ν(–NHCO), 1779 (–C=O β-lactam), 1668 (–C=O), 1608 (–C=C–), 1581 (N-H), 1379 (–COOH), 796 (Ar1H-NMR $CDCl_3$(δ); 8.18 (s, 1H, –CONH), 6.45 (t, J=10.0, 1H, H-10), 7.14 (dd, J=7.9, 2.0 Hz, 2H, H-3″, H-5″), 7.09 (dd, J=7.2, 1.9 Hz, 2H, H-2″), 5.5 (t, J=5.0 Hz, 1H, H-7), 4.9 (d, J=11.5, 2H, H-11), 4.79 (d, J=6.0 Hz, 1H, H-6), 3.4 (q, J=7.1 Hz, 1H, H-2′), 2.9 (s, 2H, H-4), 2.28 (d, J=6.9 Hz, 2H, H-7″), 1.7 (m, 1H, H-8″), 1.3 (d, J=7.0 Hz, 3H, H-3′), 0.89 (d, J=6.6 Hz, 6H, H-9″, H-10″); 13C-NMR (δ): 171.8 (C-1′), 166.5 (C-8), 162 (C-9), 140.82 (C-1″), 140 (C-9, C-10), 139 (C-3), 128.81 (C-2″), 127.37 (C-4″), 106.4 (C-2), 75.9 (C-6), 53.4 (C-2′), 45.11 (C-7), 44.93 (C-7″), 30.07(C-8″), 30.12 (C-3′), 30.07 (C-4), 22.3 (C-9″, C-10″); ESI-MS: 415.1686 [M^+H^+], 414.1688; $C_{22}H_{26}N_2O_4S$.



(A18)

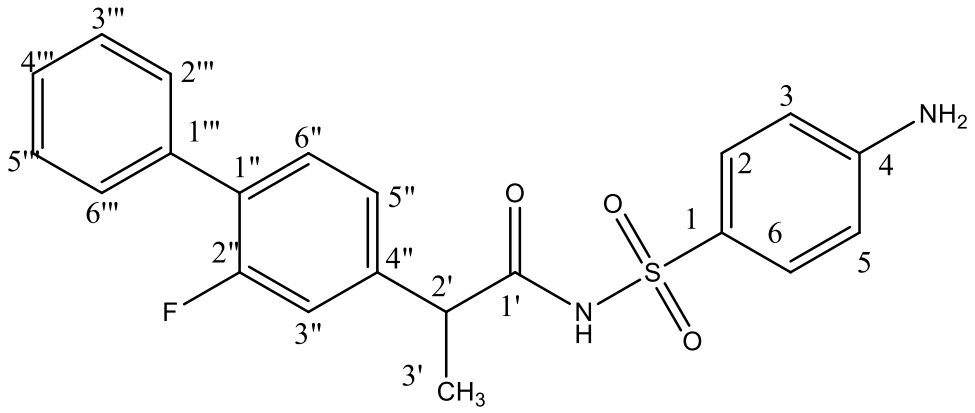
2.3.15. Synthesis of flurbiprofen chloride, 2-(2-fluoro-[1, 1′-biphenyl]-4-yl) propanoyl chloride [113]

Solution of flurbiprofen (1.1500 g, 4.7 mmol) was prepared in dry toluene (25 mL). To this dry DMF (0.03 mL) was added as catalyst. This was followed by drop wise addition of oxalyl chloride (3 mL) as the chlorinating agent; during the addition temperature was maintained at 0-5 ºC. Reaction mixture was stirred for overnight. The solvent was evaporated on a rotary evaporator. Pure flurbiprofen acid chloride was obtained. ^{1}H-NMR: 7.72, (dd, J=7.8, 2.2 Hz, H-6′), 7.55- 7.41 (m, 5H, H-2″, H-3″, H-4″, H-5″, H-6″), 7.12 (dd, J=7.8, 2.4 Hz, H-5′), 6.89 (d, J=2.30 Hz, H-3′), 3.81 (q, J=6.9 Hz, H-2), 1.54 (d, J=6.8 Hz, H-3); ^{13}C-NMR: 173.6 (C-1), 159.2 (C-2′), 136.8 (C-4′), 136.5 (C-1″), 130.0 (C-6′), 129.2 (C-5″), 127.9 (C-6″), 127.6 (C-4″), 125.7 (C-5′), 117.1 (C-3′), 55.7 (C-2), 16.4 (C-3).



2.3.16. Synthesis of prodrug of flurbiprofen and sulfanilamide, N-((4-aminophenyl) sulfonyl)-2-(2-fluoro-[1, 1'-biphenyl]-4-yl) propanamide (A6)

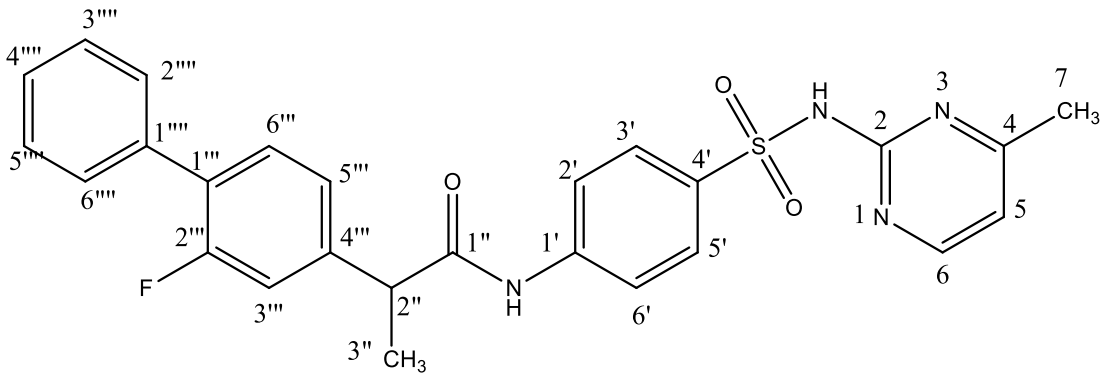
This compound was synthesized as per procedure for A20 above by replacing aspirin chloride by flurbiprofen chloride (113 mg, 0.43 mmol). Quantity of sulfanilamide taken for the reaction was (69 mg, 0.40 mmol). The purified product (A6) was then characterized. Yield: 109 mg (63.5%) ; λ_{max} = 270 nm; IR (KBr) ν cm^{-}3070 (=CH), 2864 (–CH), 1693 (–C=O amide), 1597 (–NH), 1323 (–CF), 1151 (–S=O), 842 (Ar); ^{1}H-NMR $CDCl_3$(δ); 7.81 (d, J=8.1 Hz, 1H, H-6″), 7.52 (m, 2H, H-2‴), 7.51 (m, 2H, H-3‴, H-5‴), 7.50 (d, J=2.10Hz, 1H, H-3″), 7.45 (dd, J=8.1, 2.2 Hz, 1H,1H, H-5″),7.41 (m, 1H, H-4‴), 7.28 (dd, J=8.2, 2.1 Hz, 2H, H-2, H-6), 6.65 (dd, J=8.2, 1.9 Hz, 2H, H-3, H-5), 5.55 (s, 1H, –CONH), 4.9 (s, 1H, NH_2), 3.65 (q, J=7.1 Hz, 1H, H-2′), 1.35 (d, J=7.0 Hz, 3H, H-3′); ^{13}C-NMR (δ): 172.8 (C-1″), 154.2(C-2″), 151.6 (C-4), 150.4 (C-4″), 142.6(C-1), 138.5 (C-1‴), 129.2 (C-3‴), 128.5 (C-1″), 127.9 (C-2‴), 127.1 (C-6″), 126.5 (C-4‴), 125.0 (C-5″), 124.9 C-2), 115.6 (C-3″), 115.5 (C-3, C-5), 46.0 (C-2′), 17.6 (C-3′); ESI-MS: 421.0993 [M+Na^{+}], 398.0980; $C_{21}H_{19}FN_2O_3S$.



(A6)

2.3.17. Synthesis of prodrug of flurbiprofen and sulfamerazine, 2-(2-fluoro-[1, 1'-biphenyl]-4-yl)-N-(4-(N-(4-methylpyrimidin-2-yl) sulfamoyl) phenyl) propanamide (A7)

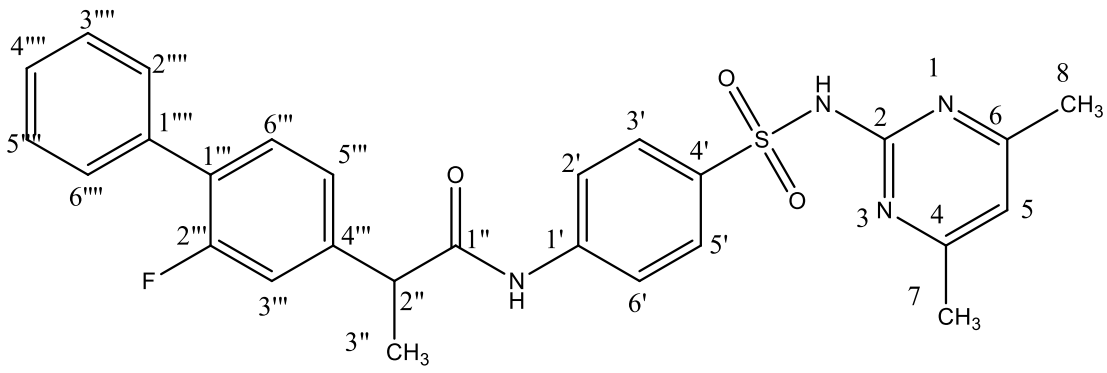
This compound was synthesized as per procedure for A20 above by replacing aspirin chloride by flurbiprofen chloride (139 mg, 0.50 mmol) and sulfanilamide by sulfamerazine (132 mg, 0.50 mmol). The purified product (A7) was then characterized. Yield: 181 mg (69.6%) ; λ_{max} 285 nm; IR (KBr) ν cm: 3251(amide), 1658 (–C=O), 1583 (–NH), 1504 (–CH), 1348 (–CN), 1249 (–CF), 1161 (–S=O), 891 (–Ar); ^{1}H-NMR $CDCl_3$(δ); 9.2 (s, 1H, –CONH), 8.3 (d, J=8.1 Hz, 1H, H-6),6.95(s, 1H), 7.52-7.48 (m, 5H, H-2‴′, H-3‴′, H-4‴′, H-5‴′, H-6‴′), 7.42 (dd, J=7.8, 2.2 Hz, 1H, H-6‴), 7.24 (dd, J=8.2, 2.5 Hz, 2H, H-6′), 7.20 (d, J=2.20 Hz, 1H, H-3‴), 7.19 (dd, J=8.2, 2.2 Hz, 2H, H-3′, H-5′), 7.15 (dd, J=7.8, 2.2 Hz, 1H, H-5‴), 6.6 (d, J=8.1Hz, 1H, H-5), 3.7 (q, J=6.9 Hz, 1H, H-2″), 2.4 (s, 3H, H-7), 1.5 (d, J=6.8 Hz, 3H, H-3″) ; ^{13}C-NMR (δ): 172.2 (C-1), 169 (C-2) 160(C-4), 159 (C-6), 157.1(C-2‴), 159 (C-6), 169.9 (C-2), 136.8 (C-4‴), 135.3 (C-4′), 135.1 (C-1‴′), 131.2 (C-1′), 129.2 (C-3‴′), 128.8 (C-1‴), 127.9 (C-2‴′, C-6‴′), 127.8 (C-6‴), 127.6 (C-4‴′), 123.5 (C-2′), 123.4 (C-5‴), 117.1 (C-3‴), 115.4 (C-3′), 115.2 (C-5), 47.5 (C-2″), 24.0 (C-7), 18.5 (C-3″); ESI-MS: 491.2190 [M+H$^+$], 490.2183; $C_{26}H_{23}FN_4O_3S$.



(A7)

2.3.18. Synthesis of prodrug of flurbiprofen and sulfamethazine, N-(4 (N-(4, 6-dimethylpyrimidin-2-yl) sulfamoyl)phenyl)-2-(2-fluoro-[1,1'-biphenyl]-4-yl) propanamide (A8)

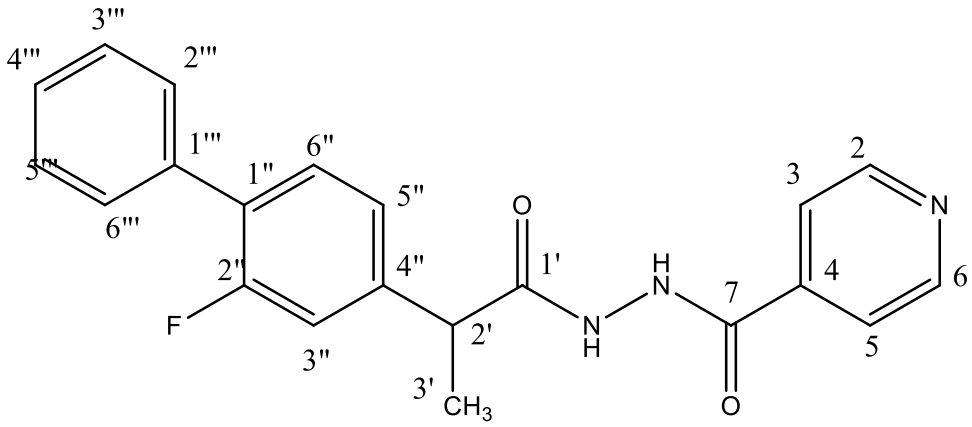
This compound was synthesized as per procedure for A20 above by replacing aspirin chloride by flurbiprofen chloride (139 mg, 0.50 mmol) and sulfanilamide by sulfamethazine (139 mg, 0.50 mmol). The purified product (A8) was then characterized. Yield: 189 mg (70.7%) ; λ_{max} 295 nm; IR (KBr) ν cm: 3334 (amide), 1680 (–C=O), 1584 (–NH), 1414 (–CF), 1153 (–S=O), 831 (–Ar); ^{1}H-NMR $CDCl_3$(δ); 8.2 (s, 1H, –SNH), 7.98 (s, 1H, –CONH), 7.55 (s, 1H, H-5), 7.52 (m, 2H, H-2‴), 7.51 (m, 2H, H-3‴), 7.48 (dd, J=8.2, 2.5 Hz, 1H, H-6‴) 7.41 (m, 1H, H-4‴), 7.25 (d, J=8.2, 2.5Hz, 2H, H-2′, H-6′), 7.20 (d, J=2.20 Hz, 3H, H-3′, H-5′, H-3‴), 7.15 (dd, J=7.8, 2.4 Hz, 1H, H-5‴), 6.7 (s,1H), 3.88 (q, J=6.9 Hz, 1H, H-2″), 2.38, (s, 6H, H-7, H-8), 1.25 (d, J=6.8 Hz, 3H, H-3″); ^{13}C-NMR (δ): 172.4 (C-1″), 169.1 (C-6), 150.9 (C-2), 136.8 (C-4‴), 135.3 (C-4′), 135.1 (C-1‴), 131.6 (C-1′), 129.2 (C-5‴), 128.8 (C-1‴), 127.9 (C-6‴), 127.8 (C-6‴), 127.6 (C-4‴), 123.8 (C-2′), 123.5 (C-6′), 123.4 (C-5‴), 117.1 (C-3‴), 115.4 (C-5′), 107.5 (C-5), 47.5 (C-2″), 24.2 (C-7, C-8), 18.5 (C-3″); ESI-MS: 505.1367 [M+H], 504.1350; $C_{27}H_{25}FN_4O_3S$.



(A8)

2.3.19. Synthesis of flubiprofen and isoniazid, N'-(2-(2-fluoro-[1, 1'-biphenyl]-4-yl) propanoyl) isonicotinohydrazide (A9)

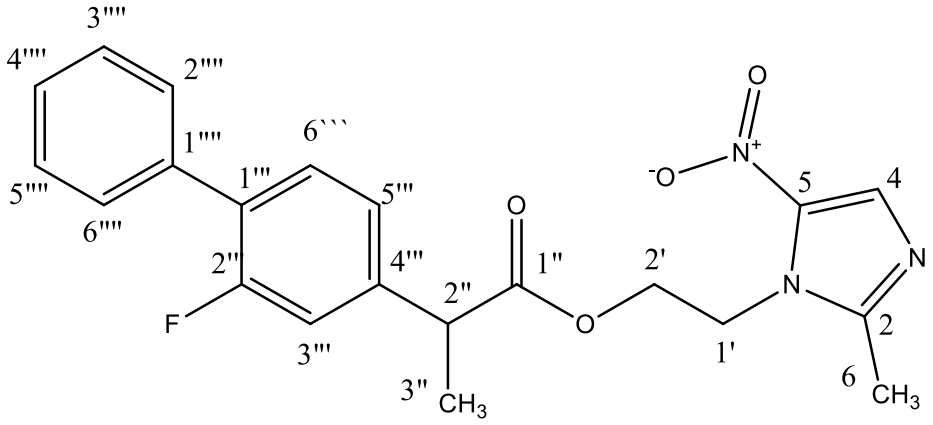
This compound was synthesized as per procedure for A4 above by replacing ibuprofen chloride with flurbiprofen chloride (210mg, 0.80 mmol). The purified product (A9) was then characterized. Yield:211 mg (72%) ; λ max 270 nm; IR (KBr) ν cm-1: 3468 (amide), 1595 (–C=O), 1323 (–CN), 1143 (–CF), 836 (–Ar); 1H-^{1}H-NMR $CDCl_3$(δ); 8.8 (dd, J=7.9, 2.1 Hz, 2H, H-2, H-6), 8.2 (br s, 2H, –CONH), 7.52 (m, 2H, H-2‴, H-6‴), 7.51 (m, 2H, H-3‴, H-5‴), 7.48 (dd, J=7.8, 2.2 Hz, 1H, H-6″), 7.41 (m, 1H, H-4‴), 7.20 (d, J=2.20 Hz, 1H, H-3″), 7.15 (dd, J=7.8, 2.4 Hz, 1H, H-5″), 3.8 (q, J=6.9 Hz, 1H, H-2′), 1.55 (d, J=6.8 Hz, 3H, H-3′); ^{13}C-NMR (δ): 172.2 (C-1′), 171.2 (C-7), 169.0 (C-2″), 150.1 (C-2), 143.1 (C-4), 136.8 (C-4″), 135.1 (C-1‴), 129.2 (C-5‴), 128.8 (C-1″), 127.8 (C-6″), 127.6 (C-4‴), 127.2 (C-2‴), 123.4 (C-5″), 123.1 (C-5), 117.1 (C-3″), 47.5 (C-2′), 18.5 (C-3′); ESI-MS: 364.1456 [M+H^{+}], 365.1446; $C_{21}H_{18}FN_3O_2$.



(A9)

2.3.20. Synthesis of prodrug of flurbiprofen and metronidazole, 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethyl 2-(2-fluoro-[1, 1'-biphenyl]-4-yl) propanoate (A13)

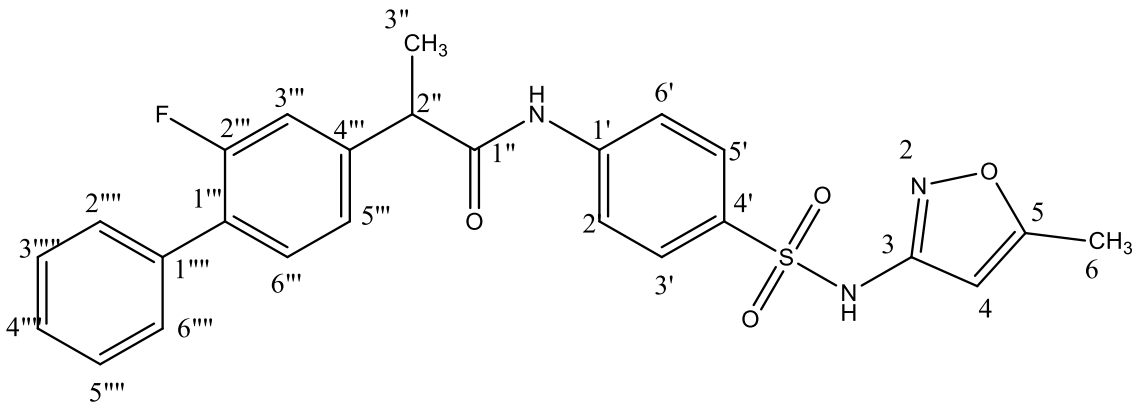
This compound was synthesized as per procedure for A4 above by replacing isoniazid with metronidazole (115 mg, 0.70 mmol) and ibuprofen chloride by flurbiprofen chloride (183 mg, 0.70 mmol). The purified product (A13) was then characterized. Yield: 193 mg (69%) ;λ_{max} 310 nm; IR (KBr) ν cm^{-1}: 1737 (–C=O ester), 1641 (–C=C–), 1529 ($–NO_2$), 1463 ($–CH_2$), 1365 ($–CH_3$), 1261 (–C(O)C–), 1178 (–CF), 1072 (–CN), 869 (–Ar); ^{1}H-NMR $CDCl_3$(δ); 8.2 (br,s, 1H, H-1″), 8.0 (s, 1H, H-4), 7.55 (m, 1H, H-6‴), 7.54 (m, 1H, H-2‴), 7.53 (m, 1H, H-3‴), 7.48 (dd, J=7.8, 2.2 Hz, 1H, H-6″), 7.41 (m, 1H, H-4‴), 7.20 (d, J=2.2 Hz, 1H, H-3″), 7.15 (dd, J=7.8, 2.4 Hz, 1H, H-5″), 4.45 (t, J=5.5 Hz, 2H, H-2′), 4.15 (t, 5.5 Hz, 2H, H-1′), 3.88 (q, J=6.9 Hz, 1H, H-2″), 2.56 (s, 3H, H-6), 1.25 (d, J=6.8 Hz, 3H, H-3″); ^{13}C-NMR (δ): 172.6 (C-1″), 169.5 (C-2), 169.0 (C-2‴), 141.5 (C-5), 136.8 (C-4), 129.2 (C-3‴, C-5‴), 128.8 (C-1‴), 127.8 (C-6‴), 127 (C-2‴, C-6‴), 123.4 (C-5‴), 122.2 (C-4‴), 117.1 (C-3‴), 63.9 (C-2′), 47.5 (C-2″), 45.6 (C-1′), 18.5 (C-3″), 15.5 (C-6); ESI-MS: 398.1511 [M+H^+], 397.1506; $C_{21}H_{20}FN_3O_4$.



(A13)

2.3.21. Synthesis of prodrug of flurbiprofen and sulfamethoxazole, 2-(2-fluoro-[1, 1'-biphenyl]-4-yl)-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl) phenyl) propanamide (A14)

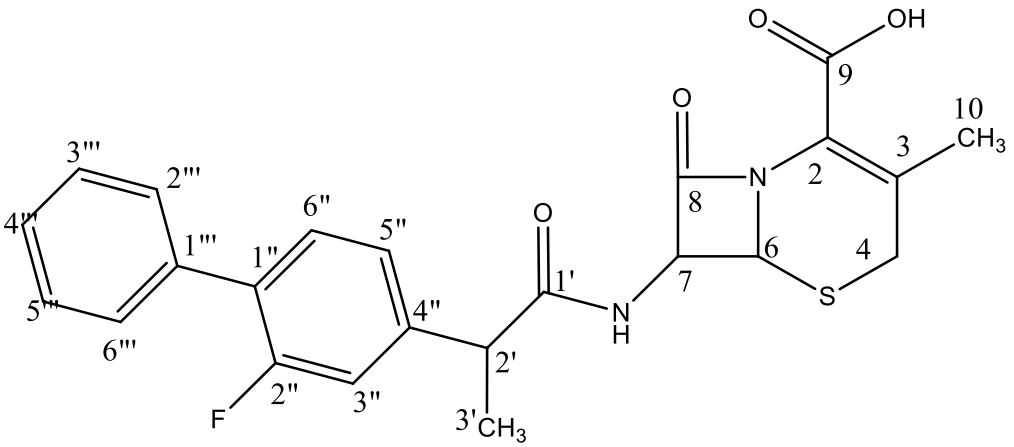
This compound was synthesized as per procedure for A20 above by replacing aspirin chloride by flurbiprofen chloride (139 mg, 0.50 mmol) and sulfanilamide by sulfamethoxazole (202.4 mg, 0.80 mmol). The purified product (A14) was then characterized. Yield 168 mg (66%); λ max 245 nm; IR (KBr) ν cm-1: 3265 (amide), 1681 (–C=O amide), 1600 (–NH), 1388 (–CF), 1259 (–CON), 1165 (–S=O), 920 (–CO), 819 (Ar); ^{1}H-NMR $CDCl_3$(δ); 8.2 (br.s. 1H, –CONH), 7.55 (m, 1H, H-6‴), 7.54 (m, 1H, H-2‴), 7.53 (m, 2H, H-5‴), 7.48 (dd, J=7.8, 2.2 Hz, 1H, H-6″),7.45 (dd, J=7.8, 2.2 Hz, 2H, H-3′, H-5′), 7.44 (dd, J=7.8, 2.1 Hz, 2H, H-2′), 7.41 (m, 1H, H-4‴), 7.20 (d, J=2.2 Hz, 1H, H-3″), 7.15 (dd, J=7.8, 2.4 Hz, 1H, H-5″), 6.9 (s, 1H, –SNH), 5.55 (s, 1H, H-4), 3.88 (q, J=6.9 Hz, 1H, H-2″), 2.25 (s, 3H, H-6), 1.25 (d, J=6.8Hz, 3H, H-3″); ^{13}C-NMR (δ): 177.1 (C-3), 172.4 (C-1″), 170.2 (C-5), 169.0 (C-2‴), 142.6 (C-1′), 141.5 (C-4′), 136.8 (C-1‴), 129.2 (C-3‴, C-5‴), 128.8 (C-1‴), 127.8 (C-6‴), 127 (C-2‴, C-6‴), 126.8 (C-3′), 123.4 (C-5″), 122.2 (C-4‴), 117.1 (C-3″), 112.5 (C-6′), 89.1 (C-4), 47.5 (C-2″), 18.5 (C-3″), 13.1 (C-6); ESI-MS: 502.1207 [M+Na^+], 479.1198; $C_{25}H_{22}FN_3O_4S$.



(A14)

2.3.22. Synthesis of prodrug of flurbiprofen and 7-ADCA, 7-(2-(2-fluoro-[1, 1'-biphenyl]-4-yl) propanamido) -3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (A16).

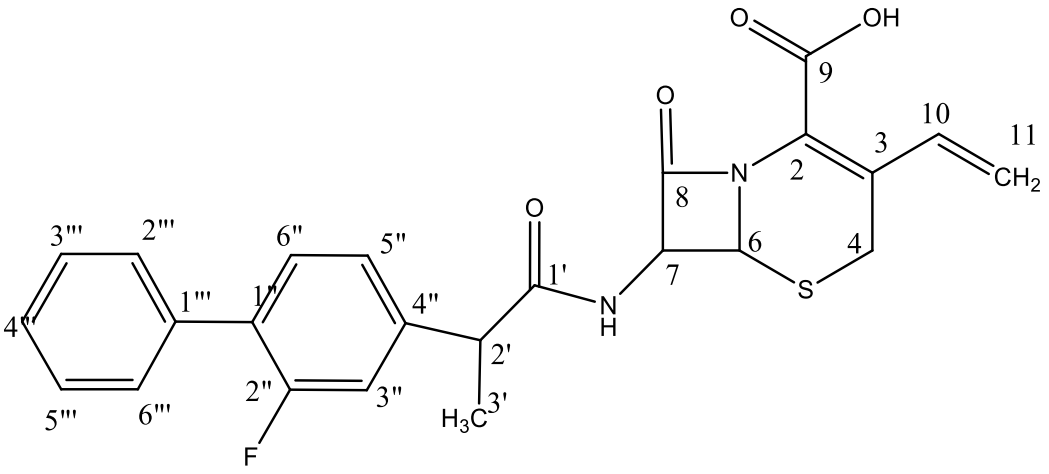
This compound was synthesized as per procedure for A4 above by replacing isoniazid with 7-ADCA (150 mg, 0.70 mmol) and ibuprofen chloride by flurbiprofen chloride (183 mg, 0.70 mmol). The purified product (A16) was then characterized. Yield: 226 mg(70%) ; λ_{max} UV-Vis. λ max 238 nm; IR (KBr) ν cm^{-1}: 3468 (–OH carboxylic group), 1775 (–C=O β-lactam), 1681(–C=O amide) 1595 (–NH), 1085 (–CF), 836 (–Ar); ^{1}H-NMR $CDCl_3$(δ); 8.22 (s,1H, –COOH), 8.1 (m, 1H, –CONH), 7.52 (m, 2H, H-2‴, H-6‴), 7.51 (m, 2H, H-3‴, H-5‴), 7.45 (dd, J=7.8, 2.2 Hz, 1H, H-6″), 7.41 (m,1H, H-4‴), 7.25 (d, J=2.20 Hz, 1H, H-3″), 7.10 (dd, J=7.8, 2.4 Hz, 1H, H-5″), 6.6 (m, 1H, H-7), 5.1 (d, J=5.1 Hz, 1H, H-6), 3.81(q, J=6.9 Hz, 1H, H-2′), 3.7 (s, 2H, H-4), 2.45 (s, 3H, H-10), 1.5 (d, J=6.8 Hz, 3H, H-3′); ^{13}C-NMR (δ): 179.8 (C-9) , 174.5 (C-8), 172.2 (C-1′), 169.0 (C-2″), 152.0 (C-4″), 136.8 (C-1‴), 129.2 (C-3‴, C-5‴), 128.9 (C-2), 128.8 (C-1″), 127.8 (C-6″), 127 (C-2‴, C-6‴), 125.1 (C-3), 123.5 (C-4‴), 123.4 (C-5″), 117.1 (C-3″), 65.5 (C-7), 52.5 (C-6), 47.5 (C-2′), 30.7 (C-4), 18.5 (C-3′), 14.2 (C-10); ESI-MS: 441.1279 [$M+H^+$], 440.1276; $C_{23}H_{21}FN_2O_4S$.



(A16)

2.3.23. Synthesis of prodrug of flurbiprofen and 7-AVCA, 7-(2-(2-fluorobiphenyl-4-yl) propanamido)-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (A17)

This compound was synthesized as per procedure for A4 above by replacing isoniazid with 7-AVCA (158 mg, 0.70 mmol) and ibuprofen chloride by flurbiprofen chloride (183 mg, 0.70 mmol). The purified product (A17) was then characterized. Yield 213 mg (67.6%) ; λ_{max} 250 nm; IR (KBr) ν cm^{-1}: 3400(–OH carboxylic group), 3180 (amide), 1779 (–C=O β-lactam), 1670 (–C=O), 1595 (–NH), 1373 (–COOH), 1031 (–CF), 770 (–Ar); ^{1}H-NMR $CDCl_3$(δ); 8.15 (s, 1H, –COOH), 7.69 (s, 1H, –CONH), 7.55 (m, 1H, H-6‴), 7.54 (m, 1H, H-2‴), 7.53 (m, 2H, H-3‴), 7.48 (dd, J=7.8, 2.2 Hz, 1H, H-6″), 7.43 (m, 1H, H-10), 7.41 (m, 1H, H-4‴), 7.20 (d, J=2.20 Hz, 1H, H-3″), 7.15 (dd, J=7.8, 2.4 Hz, 1H, H-5″), 6.61 (d, J=11.5 Hz, 2H, H-11), 5.05 (t, J=5.0 Hz, 1H, H-7), 4.85 (d, J=6.0 Hz, 1H, H-6), 3.89 (q, J=7.1Hz, 1H, H-2′), 3.77 (s, 2H, H-4), 1.25 (d, J=7.0 Hz, 3H, H-3′); ^{13}C-NMR (δ): 179.98 (C-9), 174.5 (C-8), 172.0 (C-1′), 169.0 (C-2″), 152.0 (C-4″), 135.1 (C-1‴), 129.2 (C-3‴, C-5‴), 128.8 (C-1″), 127.8 (C-6″), 127.3 (C-11), 127.1 (C-10), 127 (C-2‴, C-6‴), 123.9 (C-3), 123.5 (C-4‴), 123.4 (C-5″), 117.1 (C-3″), 123.69 (C-2), 52.75 (C-6), 53.4 (C-7), 45.42 (C-2′), 239.29 (C-4), 19.3 (C-3′); ESI-MS: 451.1686 [M+H^+], 452.1688; $C_{24}H_{21}FN_2O_4S$.

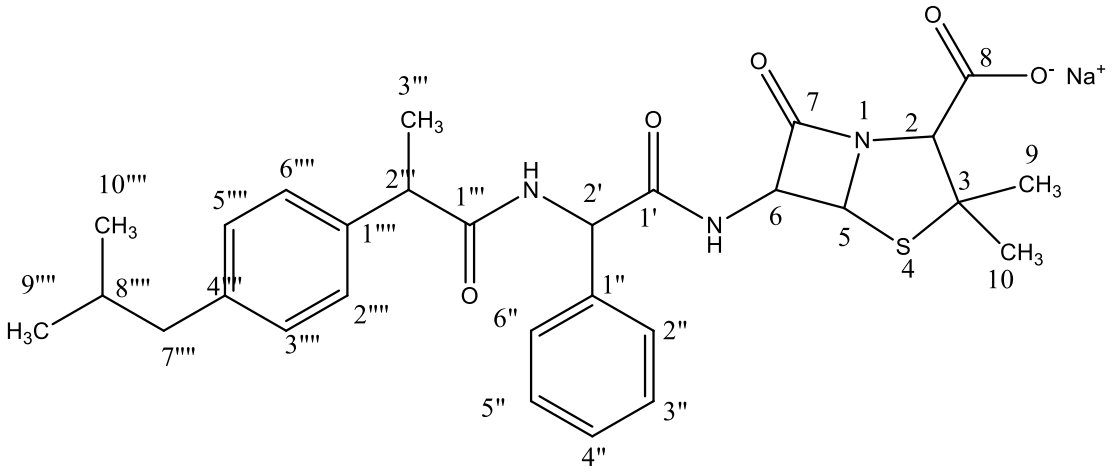


(A17)

2.3.24. Synthesis of prodrug of ibuprofen and ampicillin sodium, 6-(2-(2-(4-isobutylphenyl) propanamido)-2-phenylacetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylate (A1)

This reaction was planned according to the method given in literature [114] with slight modification. The solution of ampicillin (35 mg, 0.1 mmol) was prepared in acetonitrile (CH_3CN, 15 mL). DCCI (20 mg, 0.1 mmol) and a catalytic amount of DMAP (2.5 mg, 0.02 mmol) were added in ampicillin solution. Solution of ibuprofen (20.6 mg, 0.1 mmol) in acetonitrile (CH_3CN, 15 mL) was prepared and added to first solution at 0 ^oC. The mixture was stirred first at 0 ^oC for 15 min then at about 25 ^0C for 24 h. The reaction mixture was filtered to remove dicyclohexyl urea (DCU), the residues were washed with CH_3CN (25 mL), and the combined filtrates were evaporated in vacuum. Product was purified using flash column chromatography. TLC (pre-coated silica gel GF-254, 0.5 mm thick, Merck; 1:1 ethyl acetate: acetonitrile): 30 mg (54.4%) yellowish coloured solid(**A1**) ; λ_{max} = 300 nm; IR (KBr) ν cm^{-1}: 3298 (Carboxylic group), 1649 (–C=O), 1598 (–CH), 1519 (–NH–C=O), 1388 ($–CH_3–CH–CH_3$), 1130 (–CN); ^1H NMR (δ): 8.1 (m, 2H, –CONH), 7.39 (dd (J=7.9, 2.0 Hz, 2H, H-5′), 7.35(dd, J=7.2, 1.9 Hz, 1H, H-4′), 7.3 (dd, J=8.2, 2.5 Hz, 2H, H-2′′′, H-6′′′), 7.20 (m, 2H, H-6′), 7.09 (dd, j=8.2, 2.2 Hz, 2H, H-3′′′, H-5′′′), 5.55 (d J=4.5 Hz, 1H, H-6), 5.6 (s, 1H, H-2′), 4.8, (d, J=4.5Hz, 1H, H-5), 5 (s,1H), 4.44 (s, 1H, H-2), 3.8 (q, J=7.1 Hz, 1H, H-2′′), 2.40 (d J=6.6 Hz, 2H, H-7′′′), 1.89 (m, 1H, H-8′′′), 1.2 (s, 6H, H-9, H-10) 1.4 (d, J=6.9 Hz, 3H, H-3′′), 0.9 (d, J=6.6 Hz,6H, H-9′′′, H-10′′′); ^{13}C NMR ($CDCl_3$): 179.0 (C-8), 178.0 (C-1′), 177.0 (C-1′′), 174.0 (C-7), 140.5 (C-1′), 139.2 (C-1′′′), 129.3 (C-2′), 128.7 (C-3′), 127.6 (C-4′), 127.3 (C-2′′′, C-6′′′), 127.2 (C-3′′′, C-5′′′), 71 (C-2), 69 (C-5), 67 (C-3), 63 (C-6), 57.3 (C-

2‴), 60 (C-2′), 42.0 (C-7‴′), 28.64 (C-8‴′), 26.5 (C-9‴′, C-10‴′), 25.36 (C-9, C-10), 25.32 (C-3‴); ESI-MS: 560.2190 [M+H$^+$], 559.218; $C_{29}H_{34}N_3NaO_5S$

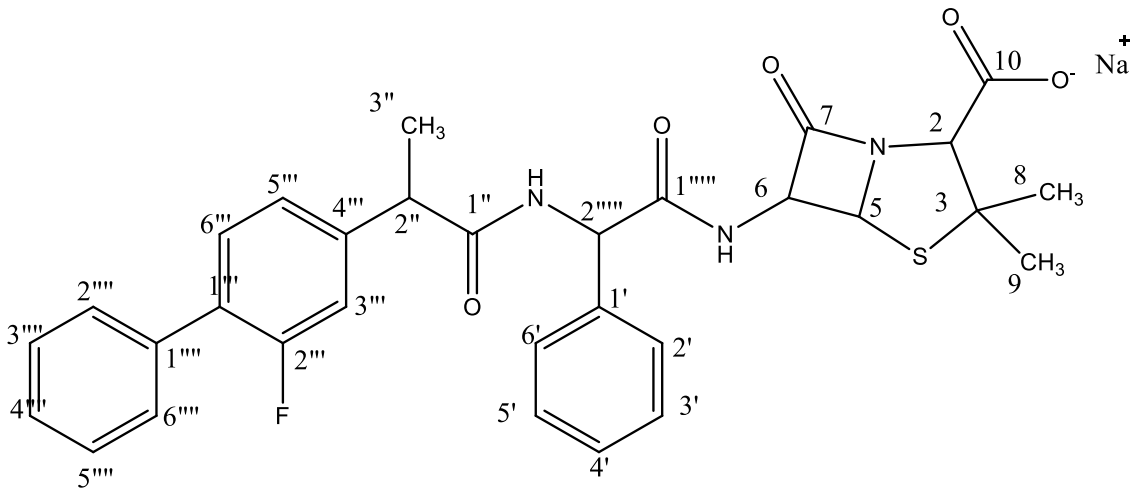


(A1)

2.3.25. Synthesis of prodrug of flurbiprofen and ampicillin sodium sodium 6-(2-(2-(2-fluoro-[1, 1'-biphenyl]-4-yl) propanamido)-2-phenylacetamido)-3, 3′-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylate (A15)

This compound was synthesized as per procedure for A1 above by replacing ibuprofen with flurbiprofen (24.4 mg, 0.1 mmol).The purified product (A15) was then characterized. Yield 35.5 mg (59.5%); λ max 290 nm; IR (KBr) ν cm-1: 3244(amide), 1774 (–C=O β-lactam), 1643 (–C=O), 1558 (–NH), 1386 (–CF), 1215 (–CO carboxylate), 808 (–Ar); ^{1}H-NMR $CDCl_3$(δ); 8.2 (br.s., 1H, –CONH), 7.55 (m, 1H, H-6‴′), 7.54 (m, 1H, H-2‴′), 7.53 (m, 2H, H-3‴′), 7.48 (dd, J=7.8, 2.2 Hz, 1H, H-6‴), 7.41 (m, 1HH-4‴′), 7.39 (dd, J=7.9, 2.0 Hz, 2H, H-3′, H-5′), 7.35 (dd, J=7.2, 1.9 Hz, 1H, H-4′), 7.20 (m, 2H, H-6′), 7.15 (dd, J=7.8, 2.4 Hz, 1H, H-5‴), 5.55 (d, J=4.5 Hz, 1H, H-6), 5.19 (s, 1H, H-1‴′′), 5.16 (d, J=4.5 Hz, 1H, H-5), 4.44 (s, 1H, H-2), 3.88 (q, J=6.9 Hz, 1H, H-2″), 1.60 (s, 6H, H-8, H-9), 1.25 (d, J=6.8 Hz, 3H, H-3″); ^{13}C-NMR (δ): 175.6 (C-10), 172.2 (C-1″), 171.5 (C-2‴′), 171.1 (C-7), 169.0 (C-2‴),

140.5 (C-1′), 136.8 (C-1‴′), 129.3 (C-2′), 129.2 (C-3‴′, C-5‴′), 128.8 (C-1‴), 128.7 (C-3′, C-5′), 127.8 (C-6‴), 127.6 (C-4′), 127 (C-2‴′, C-6‴′), 123.4 (C-5‴), 122.2 (C-4‴′), 117.1 (C-3‴), 75.9 (C-5), 75.8 (C-2), 59.6 (C-3), 58.4 (C-6), 57.2 (C-1‴″), 47.5 (C-2″), 25.3 (C-8, C-9), 18.5 (C-3″); ESI-MS: 598.1782 [M+H$^+$], 597.1776; $C_{31}H_{29}FN_3NaO_5S$.



(A15)

2.4. List of successful reactions of antibiotics and anti-inflammatory drugs

Benzydamine HCl + cefazolin sodium	(A)
Ibuprofen + ampicillin sodium	(A1)
Ibuprofen + sulfamethazine	(A2)
Ibuprofen + sulfanilamide	(A3)
Ibuprofen + isoniazid	(A4)
Ibuprofen + sulfamethoxazole	(A5)
Ibuprofen + sulfamerazine	(A10)
Ibuprofen + metronidazole	(A11)
Ibuprofen + 7-ADCA	(A12)

Ibuprofen + 7-AVCA	(A18)
Flurbiprofen + sulfanilamide	(A6)
Flurbiprofen + sulfamerazine	(A7)
Flurbiprofen + sulfamethazine	(A8)
Flurbiprofen + isoniazid	(A9)
Flurbiprofen + metronidazole	(A13)
Flurbiprofen + sulfamethoxazole	(A14)
Flurbiprofen + ampicillin sodium	(A15)
Flurbiprofen +7-ADCA	(A16)
Flurbiprofen +7-AVCA	(A17)
Aspirin + sulfanilamide	(A20)
Aspirin + sulfamethoxazole	(A24)
Aspirin + sulfamerazine	(A25)

2.5. Characterization

The intermediates and products were characterized by determination of melting points and spectroscopic techniques including FT-IR, ^{1}HNMR, ^{13}CNMR, ESI-MS and single crystal XRD.

2.5.1. Melting point

Melting points were determined in the glass capillaries using Gellenkemp melting point apparatus and are reported uncorrected.

2.5.2. Spectroscopic techniques

Electronic spectra were recorded in the UV region in methanol using Cecil 7200 spectrometer. FT-IR spectra were recorded on Thermo Nicolet, M2000 and 460

Shimadzu Spectrometer in the reflectance mode. The ^{1}H and ^{13}C-NMR spectra were recorded on Bruker AV400 and Bruker DPX 200 spectrometers respectively. The chemical shifts were reported in parts per million on the δ scale. The J values were reported in Hz. Electron impact mass spectra (ESI-MS) were recorded on Waters LCT Premier Open Access System. Chemical shifts in NMR data were reported as: singlet as s, doublet as d, triplet as t, quartet as q, multiplet as m, broad as br.

2.6. Biological Studies

2.6.1. Antibacterial activities [115, 116]

Materials

The organisms used were: *Bacillus licheniformis* (ATCC 14580), *Bacillus subtilis (ATCC 6051), Bacillus amyliquefaciens* (ATCC 23350), *Bacillus thurigenesis* (ATCC 35646*), Staphylococcus aureus* (ATCC 25923), *Escherichia coli (ATCC9637)*. The standard drugs used were: ampicillin, sulfamethazine, sulfamerazine, sulfamethoxazole, sulfanilamide, metronidazole, isoniazid, 7-ADCA and 7-AVCA.

Preparation of growth media

Nutrient broth: Bacto nutrient broth-Difco 0003 (0.8 g) was dissolved in 100 mL distilled water by heating. After adjusting the pH to 7.4, the broth was sterilized in an autoclave for 20 min at 121°C.

Nutrient agar: Bacto Nutrient broth-Difco 0003 (0.8 g) was dissolved in distilled water (100 mL) by heating. The pH was adjusted to 7.4. To this Difco 214520 agar (1.5 g) was added and the mixture was heated to obtain clear solution and sterilized in an autoclave at 121°C for 20 minutes.

Preparation of inoculum

The stock slants were cultured and loop-full of cultures was added to the sterilized slants in the test tubes. These cultures were incubated in an incubator at 37°C for 24 hours. A loop full from these cultures was transferred to the freshly prepared nutrient broth (50ml) and was incubated at 37°C for 24 hours in a shaker. These bacterial cultures served as inoculum.

Procedure

Antibacterial activity was determined by well diffusion method. All bacterial species were maintained on the nutrient agar slants. Nutrient agar was melted at 50 ^{0}C and then was mixed with 5 ml of prepared inoculums (of respective test cultures). These were then poured onto sterilized petri dishes. Lids were put on the dishes. These were allowed to cool and solidify. 5 mm diameter wells cut in the agar gel; these were 60 mm apart from each other. 100 µL (prodrug/reference standard) solutions were added into each well. The petri dishes were kept in the flat position for one and half hour and then these were incubated at 37 ^{o}C for 24 hours under aerobic conditions. Inhibition (zone) was recorded in mm. Tests were done in triplicate with prodrug and reference standard solutions. All the sample solutions were prepared at the concentration of 100µg/mL of DMSO. 100 µL of these solutions was added into each well; total quantity of the drugs added in the wells was equal to 10 µg / well. Equivalent quantity of DMSO was used as control. The results were recorded in the results and discussion section.

***Table 3**: Concentrations of antibiotics used*

Antibiotics	nmoles/well	Antibiotics	nmoles/well
Metronidazole	58	7-ADCA	47
Sulfamethazine	36	7-AVCA	44
Sulfamerazine	38	Sulfanilamide	58
Isoniazid	73	Ampicillin	27
Sulfamethoxazole	39		

***Table 4:** Concentration of prodrugs used (ibuprufen)*

Prodrugs	nmoles/well	Prodrugs	nmoles/well
A1	18	A10	22
A2	21	A11	28
A3	28	A12	25
A4	31	A18	24
A5	23		

***Table 5:** Concentration of prodrugs used (flurbiprofen)*

Prodrugs	nmoles/well	Prodrugs	nmoles/well
A6	25	A14	21
A7	20	A15	17
A8	19	A16	23
A9	28	A17	22
A13	24		

***Table 6:** Concentration of prodrugs used (aspirin)*

Prodrugs	nmoles/well
A20	30
A24	23
A25	22

2.6.2. Enzyme inhibition study

Lipoxygenase activity

Anti-inflammatory activity can be performed in vitro by different methods including Lipoxygenase (LOX) inhibition by the test compound [117,118] which was used in the present work. The LOX activity was assayed according to a reported method [119] with slight modifications. Briefly, the test compound (20 µL, 0.5 mM) was added to 100 mM phosfate buffer pH 8.0 (140 µL). To this LOX from soybean (15 µL, 600 units well^{-1}, Sigma) was added. The contents were vortex-mixed and the absorbance at 234 nm was recorded. Then the mixture was incubated at 25°C for 10 min. The reaction was initiated by the addition of substrate solution of linoleic acid (25 µL). Change in the absorbance was recorded after 6-10 min at 234 nm using Synergy HT 96-well plate reader (BioTek, USA). Baicalein (20µL, 0.5 mM) was added as a positive control in each well. For determination of IC_{50} values, solutions of the test compound were assayed at 0.5 mM, 0.25 mM, 0.125 mM, 0.0625 mM, 0.0313 mM, 0.015 mM concentrations. IC_{50} values of active compounds were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA) using the formula"

$$\text{Inhibition } (\%) = \frac{(\boldsymbol{A}\text{cont} - \boldsymbol{A}\text{ts})}{\boldsymbol{A}\text{cont}} \times 100$$

Where, ***Ac***ont = total enzyme activity without inhibitor, ***A***ts = activity in the presence of test compound.

Acetylcholinesterase assay

The Acetylcholinesterase (AChE) inhibition assay was performed according to a reported method [120] with few modifications. Briefly, the reaction mixture contained

50 mM phosfate buffer pH 7.7 (60 µL), the test compound (10 µL, 0.5 mM) and AChE (10 µL, 0.005 units) in each well. The contents were vortex-mixed and absorbance at 405 nm was recorded using Synergy HT 96-well plate reader (BioTek, USA). The mixture was then incubated for 10 min at 37ºC. The reaction was initiated by the addition of 0.5 mM acetylthiocholine iodide (10 µL) in each well as substrate. Then to each well 0.5 mM 5,5-dithiobis-(2-nitrobenzoic acid (DTNB, 10 µL) was added and, after incubation at 37ºC for 30 min, absorbance was recorded at 405 nm. Experiments were carried out in triplicate. Eserine (10 µL, 0.5 mM) was used as a positive control in each well. The percentage inhibition was calculated by the help of following equation."

$$\text{Inhibition } (\%) = \frac{(\boldsymbol{A}\text{cont} - \boldsymbol{A}\text{ts})}{\boldsymbol{A}\text{cont}} \times 100$$

Butyrylcholinesterase assay

This assay was carried out according to the procedure for AChE by replacing the enzyme with Butyrylcholinesterase (BChE) and the substrate by butyrylthiocholine bromide.

α -Chymotrypsin assay

The α-chymotrypsin inhibition activity is performed according to a reported method **[**121**]** with few modifications. Briefly, the reaction mixture contained Tris-HCl buffer pH 7.6 (60µL), the test compound (10µL, 0.5 mM) and purified α-chymotrypsin (15µL, 0.9 units) in each well. The contents were vortex-mixed and absorbance at 410 nm was recorded using Synergy HT 96-well plate reader (BioTek, USA). The mixture was then incubated for 10 min at 37ºC. The reaction was initiated by the addition of 1.3 mM N-succinyl-L-phenyl-alanine-*p*-nitroanilide, (15 µL). The change in

absorbance was recorded after 30 min at 410 nm. Experiments were carried out in triplicate. The positive and negative controls were included in the assay. Chymostatin (10 µL, 0.5 mM) was used as a standard inhibitor in each well. The percentage inhibition was calculated by the help of following equation."

$$\text{Inhibition } (\%) = \frac{(\boldsymbol{A}\text{cont} - \boldsymbol{A}\text{ts})}{\boldsymbol{A}\text{cont}} \times 100$$

IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

Free radical scavenging activity

1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of antioxidant activity according to a reported method [122] with minor modifications. Briefly, the test solution (10 µL, 0.5 mM) was added to methanolic 100 µM DPPH solution (90 µL) in 96-well plates. The contents were mixed and incubated at 37 ^{0}C for 30 min. The absorbance was measured at 517 nm using Synergy HT BioTek USA microplate reader. Quercetin (10 µL, 0.5 mM) was used as a standard in each well. All the experiments were carried out in triplicate. IC_{50} values of these drugs were calculated. A decrease in absorbance indicates increased radical scavenging activity, which was determined by the formula."

The percentage inhibition was calculated by the help of following equation.

$$\text{Inhibition } (\%) = \frac{(\boldsymbol{A}\text{cont} - \boldsymbol{A}\text{ts})}{\boldsymbol{A}\text{cont}} \times 100$$

2.6.3. Anti-tuberculosis activity

Materials

The organism used was Mycobacterium tuberculosis obtained directly from the sputum of the patients. Isoniazid was the standard drug used.

Preparation of growth media

Löwenstein–Jensen (LJ) drug-containing media were used for drug susceptibility testing (DST) of Mycobacterium tuberculosis according to the proportion method [123].In proportion method, relative growth of a definite inoculum can be determined on a drug free (control) medium while comparing it with growth on culture medium containing its critical concentration of drug used.

LJ medium is most commonly used for preparing mycobacterial culture and it is recommended by World health organisation (WHO). Components used for the preparation of this medium were mixed in the following sequence: Anhydrous monopotassium dihydrophosulfate (2.4 g), magnesium sulfate (0.24), Magnesium citrate (0.6) and L-asparagine (3.6) were dissolved in distilled water (600 mL). Glycerol (12mL) was added in to this solution. Eggs were beaten to make a homogenate. Egg homogenate (1000mL) and 2% malachite green (20 mL) were added in to the above mentioned solution. Then pH was adjusted at about 6.8. This medium (7 mL in each slope) was added in a number of slopes and was put in hot air oven at 85^{o}C for 45 min and then was left in incubator at 37^{o}C for 2 days.

As this medium was heated at 85^{o}C, during heating, egg proteins were coagulated and were solidified on cooling; that’s why no separate agar was added into the medium for preparation of the culture media.

Preparation of inoculum

Sputa of TB patients with smear grading of 2+ were used for preparing pure cultures. Sputa were digested and decontaminated using N-acetyl-L-cysteine-NaOH-Na citrate standard method [124], and concentrated by centrifugation for 15 min at 3000. Sediments were separated and re-suspended in sterile buffer (0.85%) to a volume of 1 mL. Serial dilutions of the sputum concentrate were prepared. The diluted suspensions (0.1mL) were used for inoculation. Inoculum was distributed evenly on the culture medium in growth tubes.

Procedure (Serial dilution for drug sensitivity testing, DST)

Loop full of colonies were taken from growth tubes and were added to screw capped tubes containing 5-7 glass beads with sterilized water (0.3 mL) in each tube. These were gently vortex for one minute and were then left for 10 min. Sterilized water (5 mL) was added into these and again left for 20 min. Supernatant from all tubes was taken into other labelled tubes. Turbidity was checked to be equal to 1 McFarland. 4 tubes were taken and labelled as 10^{-1}, $10^{-2,}$ $10^{-3,}$ 10^{-4}. 1 McFarland turbidity (1 mL) was taken and added in the tube labelled 10^{-1}. Distilled water (9 mL) was added in to it. It was gently mixed, then 1 mL was taken from this mixture and added to tube labelled as10^{-2}. Distilled water (9 mL) was added in this and was mixed gently. 10^{-3} and 10^{-4} were prepared similarly. Concentrations labelled as 10^{-2} and 10^{-4} were used as controls in DST. Isoniazid and the prodrugs (A4 and A9) were used at the concentration of 0.2, 0.48 and 0.5 µg mL^{-1} respectively. These were all $1.5x10^{-6}$ M solution which is actually critical concentration value for isoniazid. These solutions were then added into 10^{-2} dilution of each isoniazid, A4 and A9. These labelled tubes, containing all the test material, were incubated at 36±1 ^{o}C. Screw capped bottles were used for the purpose to allow a little gas exchange. The inoculated media was

examined after 1 week of inoculation to check any contamination. Media was allowed to be inoculated for further 5 weeks. Drug susceptibility was observed & interpreted after 6 weeks of incubation.

2.6.4. Toxicity

Acute toxicity

All the steps were performed following "OECD (Organisation for economic co-operation and development) guide lines [125] for the testing of chemicals/drugs in the animals". The animals, albino mice, were randomly selected, were marked to permit individual identification, and kept in their cages for 5 days prior to dosing. The animals were housed under natural light and dark cycles. The animals were randomly divided into three groups as control, positive control and test. Each group consisted of four animals. Mice (8-10 weeks; 25-30 g) of either sex obtained from the University animal house were used. They were allowed standard laboratory feed and water. Animals were acclimatized to this environment. The starting dose was set on 300 mg kg^{-1} body weight as no information was available for the toxicity of the synthesized compounds. The volume of the dose (as aqueous suspension) administered was 2 mL/100 g body weight. The mice were made to fast prior to dosing for 3 hours for food but not water. After that the animals were weighed and single dose of the test compound was administered using a stomach tube (gavage). After the drug administration, food was withheld for 1 h. The animals were observed individually after dosing during the first 30 min, carefully for next 4 h, and daily for total 14 days. The observations included behaviour, sleep, food and water intake, body weight and mortality.

Sub chronic toxicity

This study was carried out after getting information on acute toxicity. This involved 90 days repeated doses study to provide information on all possible health risks which can arise on repeated exposure of the test compounds over a long period of time. The test drugs were orally administered daily to three groups, control, and positive control and treated, of mice (20; 10 male, 10 female in each group) housed and fed as described for the acute toxicity test for a period of 90 days. The dose was 100 mg kg^{-1} body weight in the form of aqueous suspension (2 mL per 100 g body weight) administered by gavage. The control group received distilled water only. Observations were recorded at pre-determined intervals of time on daily basis. Body weight and behavioural changes were recorded after administration of the 7^{th} dose.

2.7. Computational analysis

All the structures of the parent drugs and mutual prodrugs were drawn in ChemDraw Ultra 12.0, optimized and saved as 'mol' files. These files were uploaded on ACD/I-Lab2. Properties like, logBB, logPS and LD_{50} were predicted by the software. LogBB is the logarithmic ratio between concentration of a drug in brain and blood. Drugs having logBB $\geq$ 0 are permeable and logBB $\leq$ 0 are considered as nearly non-permeable. LogPS, permeability surface product, determines the drug transfer across the blood-brain barrier (BBB). All these properties were recorded for the parent and the synthesized prodrugs. Other drug-like properties, including absorption, distribution (ADME), were also computed because they could not be determined experimentally due to non-availability of sufficient quantities of the synthesized compounds **[126]**.

3. Results and Discussion

Keeping in view necessity of prescribing NSAIDs concomitantly with antibiotics it is thought that the two molecules could be covalently coupled to produce a single molecule with anti-bacterial and anti-inflammatory activities. Thus some of the anti-bacterial, including sulfamethoxazole, sulfanilamide, sulfamethazine, sulfamerazine, ampicillin, isoniazid, metronidazole, 7-ADCA and 7-AVCA were coupled with some of the NSAIDs, including ibuprofen, flurbiprofen and aspirin. Four different methods were used for these synthesis, which involved preparation of acid chlorides by use of thionyl chloride (**A20, A24, A25**) and oxalyl chloride (**A2-A14, A16-A18**), use of DCCI (**A1**, **A15**), and direct interaction of the parent drug molecules (**A26**). The synthesized intermediates and final products were characterised by elemental analysis, FT-IR, electronic spectroscopy, ^{1}H and ^{13}C NMR, ESI-MS and single crystal XRD. The products were subjected to toxicity study, *in vitro* anti-bacterial testing, enzyme inhibition assays against 5-LOX, AChE, BChE and α-chymotrypsin, and anti-oxidant assay. The drug-like ADME properties were computed and the validity of these was verified by reliability index (RI) and by comparing the computed values with the experimental values from literature.

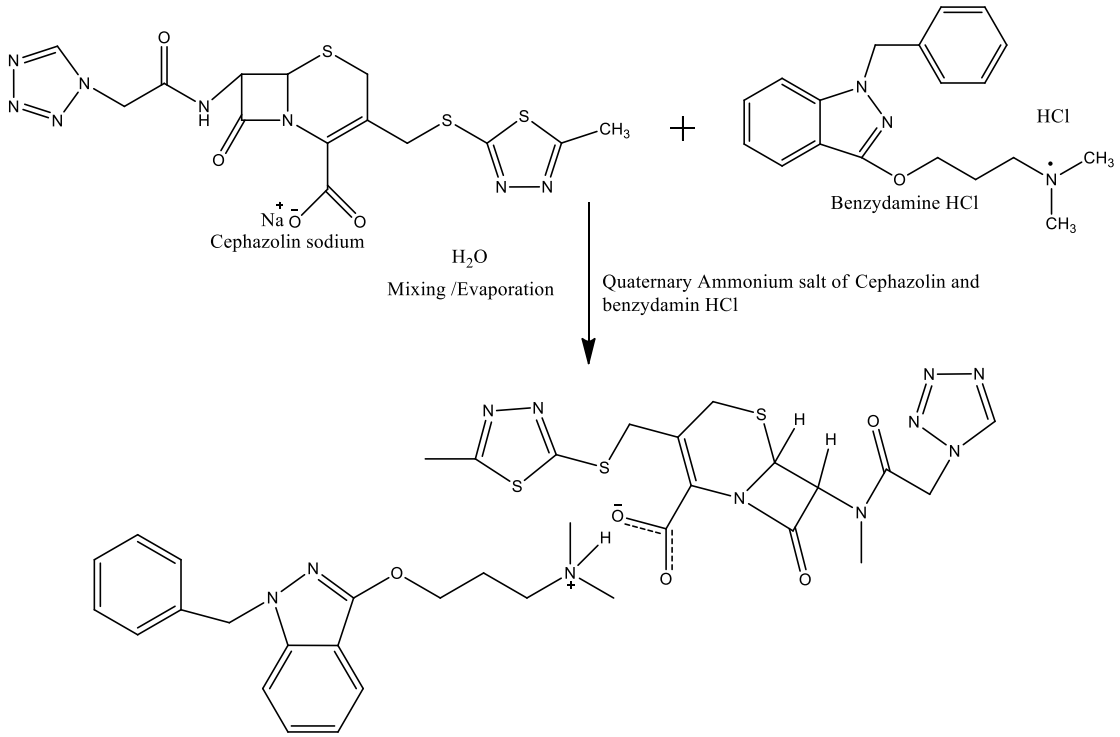
3.1. Synthesis

The NSAIDs selected for this study except benzydamine HCl contained a carboxylic acid group. The antibacterial drugs used, belong to different classes and all contained at least a primary amino group, except metronidazole. No one synthetic method was found to be suitable for synthesis of all the end products. So each synthesis is discussed separately.

3.1.1. Synthesis of benzydaminium cefazolinate

When cefazolin sodium and benzydamine HCl were mixed and stirred together in water at room temperature, a quaternary ammonium salt of the two molecules was formed, in good yield, through mutual neutralization reaction (Scheme 1). The crystalline compound was completely characterized by elemental analysis, spectroscopic data and single crystal XRD analysis.

The elemental analysis and ESI-MS results correspond to the proposed composition. In the FT-IR spectrum of the compound all the absorption bands due to cefazolin and benzydamine were present.



Scheme 1. Synthesis of benzydaminium cephazolinate

In the crystal of the molecular salt, $[C_{19}H_{24}N_3O]^+ [C_{14}H_{13}N_8O_4S_3]^-$, the cations and anions are linked by N–H- - O hydrogen bonds. Short intramolecular C–H- - O contacts occur within the anion and intermolecular C–H---O and C—H- - π bonds help to establish the packing (Figure 1).

3.1.2. Synthesis of aspirin prodrugs (A20, A24, A25)

These prodrugs were synthesized in two steps according to **Scheme 2** [106, 107]. In first step, aspirin acid chloride was synthesized by reacting aspirin with thionyl chloride in the presence of DMF as a catalyst. The product produced single spot in the TLC. The elemental analysis of the purified product gave CHN analysis conforming to $C_9H_7O_3Cl$ composition within 0.3% experimental error. The melting point and spectral data were comparable with the literature values confirming the formation of the acid chloride. The yield was good.

Synthesis of A20

Purified **A20** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new ν(–NH_2CO) band at 3230 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 3479 cm^{-1} due to ν(–NH_2) of sulfanilamide. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of a new peak at δ 7.85 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in aspirin at δ 11.95 ppm and proton of the –NH_2 in sulfanilamide at δ 5.76 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –<u>C</u>OOH signal at 170.44 ppm to a lower value of 160.8 ppm. The elemental analysis and ESI-MS (M+ m/z: 334.35) data conformed to the $C_{15}H_{14}N_2O_5S$ composition.

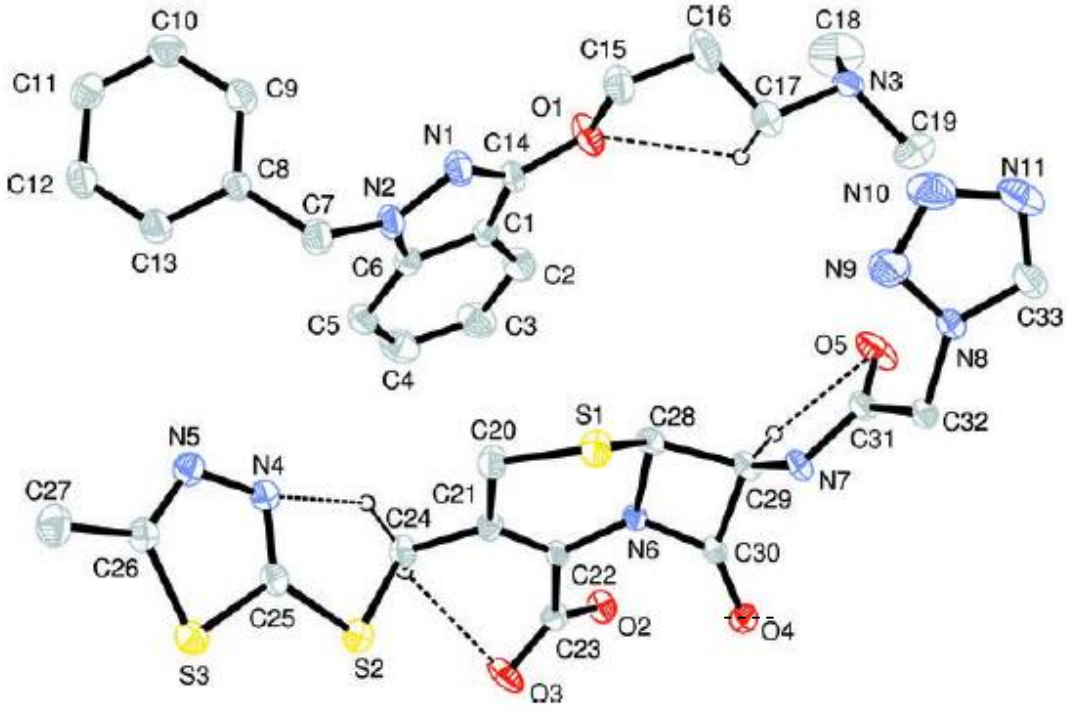


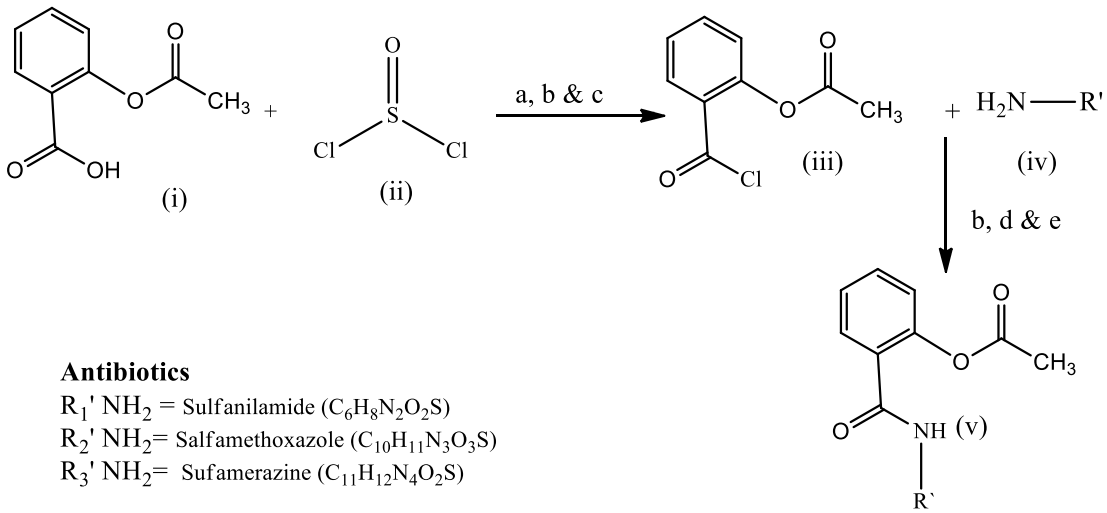
Figure 1: XRD view of benzydaminium cephazolinate

Synthesis of A24

Purified **A24** was obtained in good yield according to the experimental procedure. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of a new peak at δ 7.80 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in aspirin at δ 11.95 ppm and proton of the $–NH_2$ in sulfamethoxazole at δ 6.1 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –COOH signal at 170.44 ppm to a lower value of 160.9 ppm. The elemental analysis and ESI-MS (M+ m/z: 415.07) data conformed to the $C_{19}H_{17}N_3O_6S$ composition.

Characterization of A25

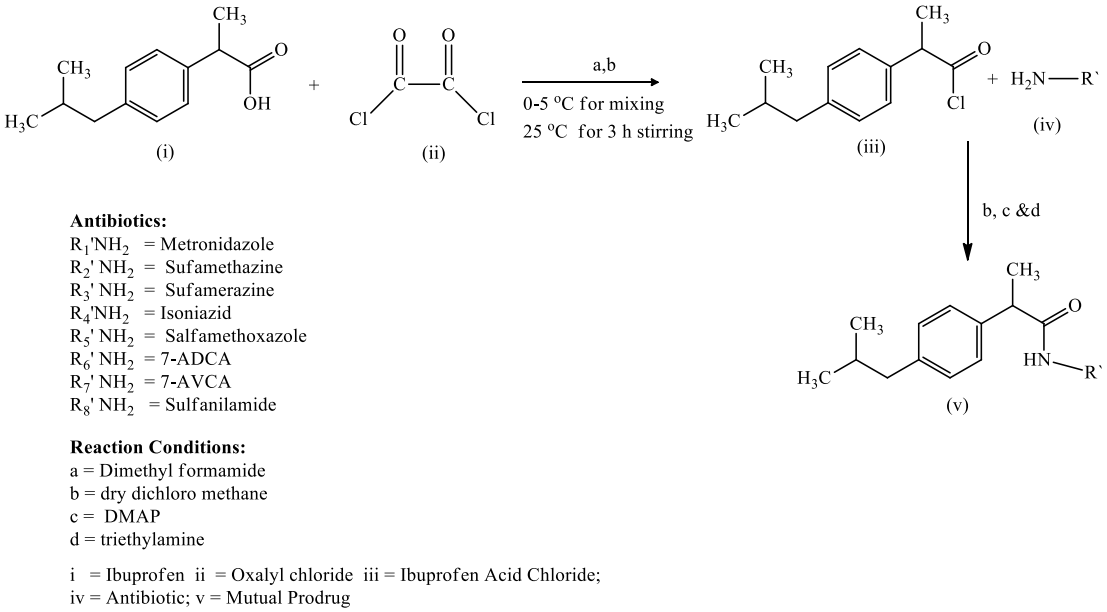
Purified **A25** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new ν($–NH_2CO$) band at 3182 cm^{-1} and 1683 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 1720 cm^{-1} due to ν($–NH_2$) of sulfamerazine. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 8.1 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in aspirin at δ 11.95 ppm and proton of the $–NH_2$ in sulfamerazine at δ 5.98 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –COOH signal at 170.44 ppm to a lower value of 160.96 ppm. The elemental analysis and ESI-MS (M+ m/z: 426.0897) data conformed to the $C_{20}H_{18}N_4O_5S$ composition.



Scheme 2- General reaction for synthesis of aspirin prodrugs (A20, A24, A25)

3.1.3. Synthesis of ibuprofen prodrugs (A2, A3, A4, A5, A10, A11, A12, A18)

These prodrugs were synthesized in two steps according to **Scheme 3** [108]. In first step, ibuprofen acid chloride was synthesized by reacting ibuprofen with oxalyl chloride in the presence of DMF as a catalyst. The product produced single spot in the TLC. The elemental analysis of the purified product gave CHN analysis conforming to $C_{13}H_{17}OCl$ composition within 0.3% experimental error. The spectral data were comparable with the literature values confirming the formation of the acid chloride. The yield was good.



Scheme 3: synthesis of ibuprofen prodrugs A2, A3, A4, A5, A10, A11, A12, A18

Synthesis of A2

Purified **A2** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new ν(–NH_2CO) band at 3334 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 3443 cm^{-1} due to ν(–NH_2) of sulfamethazine. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 8.2 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in ibuprofen at δ 11.6 ppm and proton of the –NH_2 in sulfamethazine at δ 5.98 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –COOH signal at 178 ppm to a lower value of 172.2 ppm. The elemental analysis and ESI-MS (M+ m/z: 466) data conformed to the $C_{25}H_{30}N_4O_3S$ composition,

Synthesis of A3

Purified **A3** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new ν(–NHCO) band at 3283 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 3462 cm^{-1}due to ν($–NH_2$) of sulfanilamide. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 8.22 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in ibuprofen at δ 11.6 ppm and proton of the $–NH_2$ in sulfanilamide at δ 6.86 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –COOH signal at 178 ppm to a lower value of 173 ppm. The elemental analysis and ESI-MS (M+ m/z: 360) data conformed to the $C_{19}H_{24}N_2O_3S$ composition.

Synthesis of A4

Purified **A4** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new ν(–NHCO) band at 3337 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 3430 cm^{-1} due to ν($–NH_2$) of isoniazid. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 8.9 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in ibuprofen at δ 11.6 ppm and proton of the $–NH_2$ in isoniazid at δ 4.64 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by

shifting of –COOH signal at 178 ppm to a lower value of 168.8 ppm. The elemental analysis and ESI-MS (M+ m/z: 325) data conformed to the $C_{19}H_{23}N_3O_2$ composition.

Synthesis of A5

Purified **A5** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new ν(–NHCO) band at 3219 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 3442 cm^{-1} due to ν(–NH_2) of sulfamethoxazole. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 7.9 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in ibuprofen at δ 11.6 ppm and proton of the –NH_2 in sulfamethoxazole at δ 6.1 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –COOH signal at 178 ppm to a lower value of 169.4 ppm. The elemental analysis and ESI-MS (M+ m/z: 441) data conformed to the $C_{23}H_{27}N_3O_4S$ composition.

Synthesis of A10

Purified **A10** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new (–NHCO) band at 1678 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 3400 cm^{-1} due to ν(–NH_2) of sulfamerazine. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 7.6 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in ibuprofen at δ 11.6 ppm and proton of the –NH_2 in

sulfamerazine at δ 5.98 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –COOH signal at 178 ppm to a lower value of 172.9 ppm. The elemental analysis and ESI-MS (M+ m/z: 452.57) data conformed to the $C_{24}H_{28}N_4O_3S$ composition.

Synthesis of A11

Purified **A11** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new (–COOC) band at 1734 cm^{-1} due to formation of the prodrug containing ester linkage and disappearance of the band at 3836 cm^{-1} & 1189 cm^{-1} due to ν(–OH) of metronidazole. The typical spectrum is shown in Figure 2(a). The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with disappearance of peaks of hydroxyl of the –COOH group in ibuprofen at δ 11.6 ppm and proton of the –OH in metronidazole at δ 5.03 ppm. The typical spectrum is shown in Figure 2(b). In the ^{13}CNMR formation of ester linkage was confirmed by shifting of –COOH signal at 178 ppm to a lower value of 174.5 ppm. The typical spectrum is shown in Figure 2(c). The elemental analysis and ESI-MS (M+ m/z: 359.42) data conformed to the $C_{19}H_{25}N_3O_4$ composition. The typical spectrum is shown in Figure 2(d).

Synthesis of A12

Purified **A12** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new ν(–NHCO) band at 3400 cm^{-1} due to formation of the prodrug containing amide linkage.

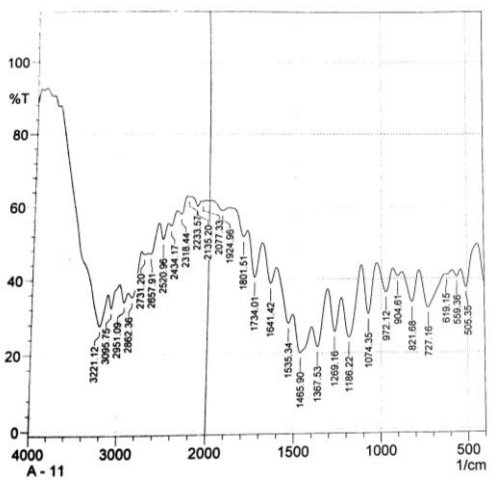


Figure 2(a)

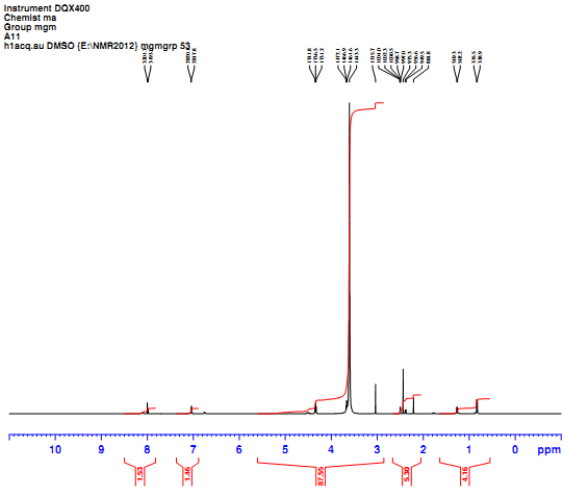


Figure 2(b)

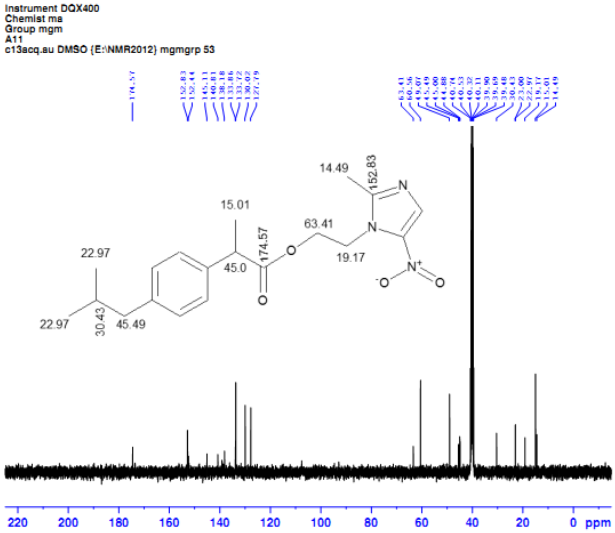


Figure 2(c)

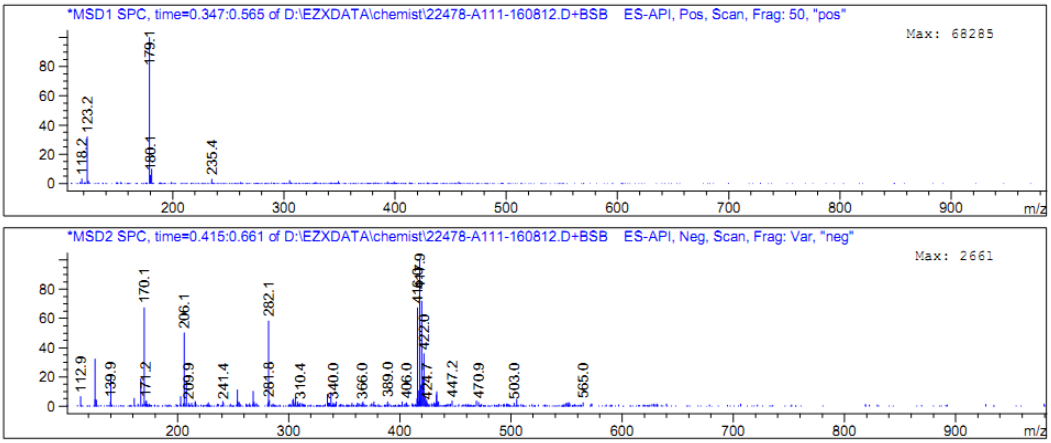


Figure 2(d)

Figure 2: FTIR, H-NHR, C13-NMR and ESI-MS spectra of A11**.**

The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 7.6 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in ibuprofen at δ 11.6 ppm and proton of the $-NH_2$ in 7-ADCA at δ 5.11 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –COOH signal at 178 ppm to a lower value of 171.8 ppm. . The elemental analysis and ESI-MS (M+ m/z: 415.16) data conformed to the $C_{22}H_{26}N_2O_4S$ composition.

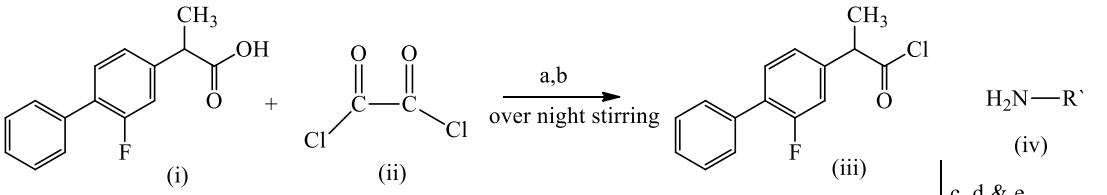
Synthesis of A18

Purified **A18** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new ν(–NHCO) band at 3270 cm^{-1} due to formation of the prodrug containing amide linkage. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 87.6 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in ibuprofen at δ 11.6 ppm and proton of the $-NH_2$ in 7-AVCA at δ 5.11 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –COOH signal at 178 ppm to a lower value of 171.8 ppm. The elemental analysis and ESI-MS conformed molecular ion peak at m/z 415.16 and also the molecular formula $C_{22}H_{26}N_2O_4S$.

3.1.4. Synthesis of flurbiprofen prodrugs (A6, A7, A8, A9, A13, A14, A16, A17)

These prodrugs were synthesized in two steps according to **Scheme 4** [109]. In first step, flurbiprofen acid chloride was synthesized by reacting flurbiprofen with oxalyl chloride in the presence of DMF as a catalyst. The product produced single spot in the TLC. The elemental analysis of the purified product gave CHN analysis

conforming to $C_{15}H_{12}FOCl$ composition within 0.3% experimental error. The yield was good. Steps for the general reaction are shown in scheme 3.



Antibiotics:
$R_1'NH_2$ = Metronidazole
$R_2'NH_2$ = Sufamethazine
$R_3'NH_2$ = Sufamerazine
$R_4'NH_2$ = Isoniazid
$R_5'NH_2$ = Salfamethoxazole
$R_6'NH_2$ = 7-ADCA
$R_7'NH_2$ = 7-AVCA
$R_8'NH_2$ = Sulfanilamide

Reaction Conditions:
a = Dimethyl formamide
b = Toluene .
c = DMAP
d = Dichloro methane
e = triethylamine

i = Flurbiprofen ii = Oxalyl chloride ; iii = Flurbiprofen acid chloride;
iv = Antibiotic; v = Mutual Prodrug

Scheme 4: synthesis of prodrugs of flurbiprofen (A6, A7, A8, A9, A13, A14, A16, and A17)

Synthesis of A6

Purified **A6** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new (–NHCO) band at 1583 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 1710 cm^{-1} due to ($–NH_2$) of sulfanilamide. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 8.2 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in ibuprofen at δ 11.6 ppm and proton of the $–NH_2$ in sulfanilamide at δ 6.86 ppm. In the ^{13}CNMR formation of amide linkage was

confirmed by shifting of –COOH signal at 178 ppm to a lower value of 172.25 ppm. The elemental analysis and ESI-MS (M+ m/z: 398) data conformed to the $C_{21}H_{19}FN_2O_3S$ composition.

Synthesis of A7

Purified **A7** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new ν(–NHCO) band at 3251 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 1710 cm^{-1} due to ($–NH_2$) of sulfamerazine. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 9.2 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in flurbiprofen at δ 11.6 ppm and proton of the $–NH_2$ in sulfamerazine at δ 5.98 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –COOH signal at 178 ppm to a lower value of 172.2 ppm. The elemental analysis and ESI-MS (M+ m/z: 490) data conformed to the $C_{26}H_{23}FN_4O_3S$ composition.

Synthesis of A8

Purified **A8** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of new bands at 3334cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of bands at 1710 cm^{-1} due to reaction of carboxylic group to produce amide linkage. The ^{1}H-NMR spectrum of the compound showed all the peaks present in the spectra of the component drugs with appearance of new peak at δ 7.98 ppm due to formation of amide group and

disappearance of peaks of hydroxyl group of the –COOH (in flurbiprofen) at δ 12.19 ppm and proton of the NH_2 (in sulfamethazine) at δ 5.98 ppm as these protons were taken part in the amide formation reaction. –CONH is at position C1″ and was proved by shifting of ^{13}C signal to a lower value from180.7 ppm to 172.4 ppm. The elemental analysis and ESI-MS (M+ m/z: 504) data conformed to the $C_{27}H_{25}FN_4O_3S$.

Synthesis of A9

Purified **A9** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new (–NHCO) band at 1595 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 1710 cm^{-1} due to ($–NH_2$) of isoniazid. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 8.2 ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in flurbiprofen at δ 11.6 ppm and proton of the $–NH_2$ in isoniazid at δ 4.64 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –<u>C</u>OOH signal at 180.7 ppm to a lower value of 178 ppm. The elemental analysis and ESI-MS conformed The ESI-MS confirmed molecular ion peak at m/z 364 and also the molecular formula $C_{21}H_{18}FN_3O_2$.

Synthesis of A13

Purified **A13** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new ν(–COOC) band at 1737 cm^{-1} due to formation of the prodrug containing ester linkage. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with

disappearance of peaks of hydroxyl of the –COOH group in flurbiprofen at δ 11.6 ppm and proton of the –OH in metronidazole at δ 5.03 ppm. In the ^{13}CNMR formation of ester linkage was confirmed by shifting of –COOH signal at 180.7 ppm to a lower value of 172.6 ppm. The elemental analysis and ESI-MS (M+ m/z: 397) data conformed to the $C_{21}H_{20}FN_3O_4$.

Synthesis of A14

Purified **A14** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new ν(–NHCO) band at 3265 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 1710 cm^{-1} due to (–NH_2) of sulfamethoxazole. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 8.2 due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in flurbiprofen at δ 11.6 ppm and proton of the –NH_2 in sulfamethoxazole at δ 6.1 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –COOH signal at 180.77 ppm to a lower value of 172.4 ppm. The elemental analysis and ESI-MS (M+ m/z: 502) data conformed to the $C_{25}H_{22}FN_3O_4S$.

Synthesis of A16

Purified **A16** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new (–NHCO) band at 1595 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 1710 cm^{-1}due to (–NH_2) of 7-ADCA. The ^{1}H-NMR spectrum of the compound

contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 8.1ppm due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in flurbiprofen at δ 11.6 ppm and proton of the $–NH_2$ in 7-ADCA at δ 5.1 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –COOH signal at 180.77 ppm to a lower value of 172.2 ppm. The elemental analysis and ESI-MS (M+ m/z: 440) data conformed to the $C_{23}H_{21}FN_2O_4S$.

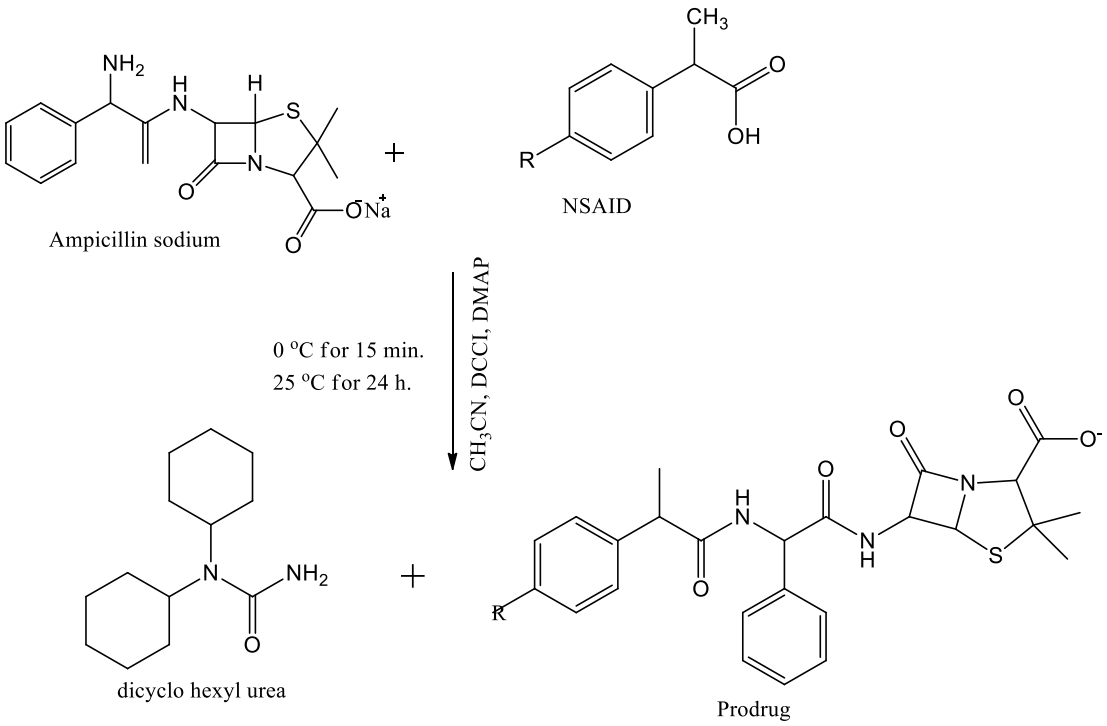
Synthesis of A17

Purified **A17** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of a new (–NHCO) band at 1595 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of the band at 1710 cm^{-1} due to ($–NH_2$) of 7-AVCA. The ^{1}H-NMR spectrum of the compound contained all the peaks in the spectra of the component drugs with appearance of new a peak at δ 8.1 due to formation of amide group and disappearance of peaks of hydroxyl of the –COOH group in flurbiprofen at δ 11.6 ppm and proton of the $–NH_2$ in 7-AVCA at δ 5.1 ppm. In the ^{13}CNMR formation of amide linkage was confirmed by shifting of –COOH signal at 180.77 ppm to a lower value of 172.2 ppm. The elemental analysis and ESI-MS conformed molecular ion peak at m/z 451 and also the molecular formula $C_{24}H_{21}FN_2O_4S$.

3.1.5. Synthesis of ibuprofen and flurbiprofen prodrugs with ampicillin (A1, A15)

These prodrugs were synthesized in single step reaction according to **Scheme** [110]. In this prodrug was synthesized by reacting ampicillin with ibuprofen/flurbiprofen in the presence of DCCI as a catalyst. The product produced single spot in the TLC. The

elemental analysis of the purified product gave CHN analysis conforming to molecular composition of both products within 0.3% experimental error.



Scheme 5: synthesis of prodrugs of ampicillin (A1, A15)

Synthesis of A1

Purified **A1** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of new bands at1519 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of bands at 1710 cm^{-1} due to reaction of carboxylic group to produce amide linkage. The ^{1}HNMR spectrum of the compound showed all the peaks present in the spectra of the component drugs with appearance of new peak at δ 8.1 ppm due to formation of amide group and disappearance of peaks of hydroxyl group of the –COOH (in flurbiprofen) at δ 11.95 ppm and proton of the NH_2 (in ampicillin) at δ 5.42 ppm as these protons were taking part in the amide formation. In the ^{13}CNMR the –CONH signal at 178 ppm shifted to

a lower value of 176 ppm. The elemental analysis and ESI-MS (M+ m/z: 559) data conformed to the $C_{29}H_{34}N_3NaO_5S$.

Synthesis of A15

Purified **A15** was obtained in good yield according to the experimental procedure. The FT-IR spectrum of the compound contained all the bands present in the spectra of the component drugs with appearance of new bands at 3244 cm^{-1} due to formation of the prodrug containing amide linkage and disappearance of bands at 1710 cm^{-1} due to reaction of carboxylic group to produce amide linkage. The ^{1}H-NMR spectrum of the compound showed all the peaks present in the spectra of the component drugs with appearance of new peak at δ 8.2ppm due to formation of amide group and disappearance of peaks of hydroxyl group of the –COOH (in flurbiprofen) at δ 12.19 ppm and proton of the NH_2 (in ampicillin) at δ 5.42 ppm as these protons were taken part in the amide formation reaction. –CONH is at position 1″ and was proved by shifting of ^{13}C signal to a lower value from180.77 ppm to172.2 ppm. The elemental analysis and ESI-MS (M+ m/z: 597) data conformed to the $C_{31}H_{29}FN_3NaO_5S$.

3.2. Biological activities

Biological activities of the prodrugs were studied by determining their anti-bacterial and enzyme inhibition activities. The results are discussed as follows.

3.2.1. Antibacterial activities

The results are given in Table 8. All the products inhibited the growth of different organisms to variable extents. Results indicated that in parent drugs, only sulfamerazine and ampicillin showed better inhibition zones against *E. coli*. In other cases all the products, along with prodrugs of these two antibiotics showed better

zones of inhibition as compared to standard antibiotics used. Flurbiprofen prodrugs (A6, A7, A16, A17, and A18) were found to be more active than aspirin prodrugs which in turn were more active than ibuprofen prodrugs. Similar trend was observed for *S. aureus*. Results of prodrugs of ampicillin, sulfamethazine, isoniazid, metronidazole sulfamethoxazole were positive against *S.aureus*. The prodrugs A3, A16 and A25 exhibited activities similar to the parent drugs against *S. aureus*. Compounds derived from 7-AVCA showed highest activities against *E. coli S. aureus*. Nearly all the synthesized prodrugs inhibited the growth of both Gram-positive and Gram-negative bacteria, showing their broad spectrum activity. The prodrugs did not show significant activities against *Bacillus thuringenesis*. Moderate activities were shown by all the prodrugs against *Bacillus amyliquefaciens* and enhanced activities were observed against *Bacillus licheniformis* as compared with those of the parent antibiotics except for A3, A10 and A17.

Table 7: Antibacterial activity of standard drugs

Compound	***E. coil***	***S. aureus***	***Bacillus subtillis***	***Bacillus amyliquefaciens***	***Bacillus licheniformis***	***Bacillus thuringenesis***
10 μg/ well	Zone of Inhibition (mm)					
Metronidazole	-	-	17	-	8	12
Sulfamethazine	-	25	-	-	-	15
Sulamerazine	28	24	-	-	-	15
Isoniazid	-	15	-	-	12	10
Sulfamthoxazole	-	38	-	13	-	16
7-ADCA	-	-	10	-	10	25
7-AVCA	-	26	-	-	-	10
Sulfanilamide	-	-	9	20	11	18
Ampilicin	50	48	22	13	11	-

Table 8: *Antibacterial activity of prodrugs*

Compound	***E. coil***	***S. aureus***	***Bacillus subtillis***	***Bacillus amyliquefaciens***	***Bacillus licheniformis***	***Bacillus thuringenesis***
10 µg/ well	Zone of inhibition (mm)					
A1	18	11	18	13	13	19
A2	19	21	23	-	18	18
A3	-	-	11	15	-	10
A4	21	20	25	19	25	18
A5	16	17	20	21	14	22
A6	22	23	12	19	12	-
A7	19	21	18	-	21	22
A8	20	28	22	21	19	-
A9	20	24	24	-	15	18
A10	22	21	-	10	-	20
A11	12	-	20	-	20	-
A12	20	28	20	-	20	-
A13	14	16	22	10	14	-
A14	-	23	15	12	15	-
A15	20	18	18	-	15	15
A16	15	-	12	-	24	25
A17	37	36	-	12	-	25
A18	42	40	-	-	26	22
A20	18	21	15	17	20	-
A24	-	29	-	20	18	23
A25	20	-	16	22	22	25

3.2.2. Enzyme inhibition studies

Anti-inflammatory assay for the synthesized prodrugs

Anti-inflammatory activity of the prodrugs was determined *in vitro* by enzyme inhibition assays as it was the other expected function of the product. The prodrugs exhibited better anti-inflammatory effect as compared with the parent NSAIDs (Table 9). Prodrugs were assayed for their inhibition against 5-LOX. The inhibition of the enzyme by ibuprofen was 42.51±0.12 % while its prodrugs (A1, A2, A10, A11, A12, A18), exhibited better activities (Table 10).

AI activity of flurbiprofen was 95.81±0.16 %; whereas, its prodrugs exhibited significant activities but lesser than the parent drug. AI activity of aspirin was 38.45±0.13 % and its prodrugs exhibited enhanced activities. The activities of A20, A24 and A25 were 2, 2.5 and 3 times more active than the aspirin, respectively.

Acetylcholinesterase assay

AChE inhibition assay was performed for all the synthesized prodrugs and was compared with the parent drugs (ibuprofen, flurbiprofen, and aspirin). The compounds A1, A2, A5, A6, A7, A8, A9, A15, A16, A18, and A24 exhibited excellent inhibitory activities and better than all the parent drugs (Table 11 and Table 12).

Butyrylcholinesterase assay

Prodrugs synthesized from ibuprofen, flurbiprofen and aspirin exhibited better results than all the three parent drugs (Table 13 and Table 14). These results are encouraging and can address the issue of dementia.

Table 9: *Anti-Inflammatory assay - standards*

Anti-inflammatory drug	**Concentration of the solution used in assay (mM)**	**Inhibition (%)**	**IC_{50} (µmol)**
Ibuprofen	0.5	42.51±0.12	_
Flurbiprofen	0.5	95.81±0.16	50.51±0.14
Aspirin	0.5	38.45±0.13	_
Baicalein	0.5	93.79±1.27	22.4±1.3

Table 10: *Anti-Inflammatory activity - prodrugs*

Sample name	**Concentration of the solution used in assay (mM)**	**Inhibition (%)**	**IC_{50} (µmol)**
A1	0.5	48.45±0.11	
A2	0.5	43.31±0.17	
A3	0.5	48.37±0.14	
A4	0.5	49.63±0.15	
A5	0.5	40.41±0.16	
A6	0.5	95.74±0.11	
A7	0.5	83.72±0.19	
A8	0.5	90.21±0.13	
A9	0.5	75.21±0.16	
A10	0.5	89.05±0.15	58.21±0.04
A11	0.5	46.43±0.14	_
A12	0.5	47.67±0.61	_
A13	0.5	55.72±0.12	<400
A14	0.5	36.24±0.17	-
A15	0.5	45.45±0.12	_
A16	0.5	68.41±0.18	111.21±0.11
A17	0.5	42.73±0.17	_
A18	0.5	58.33±0.13	234.51±0.05
A20	0.5	70.67±0.15	107.38±0.17
A24	0.5	78.11±0.13	91.25±0.16
A25	0.5	94.09±0.11	52.61±0.0.5

Table 11: *Acetylcholinesterase assay of standards*

Drugs used as standard	**Concentration of the solution used in assay (mM)**	**Inhibition (%)**	**IC_{50} (µmol)**
Ibuprofen	0.5	61.19±0.04	235.11±0.17
Flurbiprofen	0.5	31.43±0.05	50.51±0.14
Aspirin	0.5	49.59±0.11	<400
Eserine	0.25	91.29±1.17	0.04±0.0001

Table 12: *Acetylcholinesterase assay of prodrugs*

Prodrug code	**Concentration of the solution used in assay (mM)**	**Inhibition (%)**	**IC_{50} (µmol)**
A1	0.5	71.95±0.22	143.61±0.04
A2	0.5	74.27±0.32	138.41±0.11
A3	0.5	47.21±0.11	<600
A4	0.5	56.39±0.76	<400
A5	0.5	85.31±0.88	81.61±0.17
A6	0.5	69.05±0.19	201.21±0.17
A7	0.5	70.15±0.78	198.65±0.25
A8	0.5	72.34±0.43	139.51±0.21
A9	0.5	78.92±0.24	112.11±0.14
A10	0.5	66.73±0.62	219.41±0.04
A11	0.5	63.83±0.35	223.11±0.01
A12	0.5	67.51±0.33	196.31±0.03
A13	0.5	63.83±0.68	251.23±0.22
A14	0.5	50.21±0.58	<400
A15	0.5	73.69±0.98	127.31±0.11
A16	0.5	71.48±0.56	126.91±0.11
A17	0.5	69.65±0.36	131.25±0.14
A18	0.5	71.39±0.21	125.21±0.05
A20	0.5	44.94±0.14	<600
A24	0.5	72.39±0.16	115.71±0.16
A25	0.5	47.11±0.18	<600

Table 13: *Butyrylcholinesterase assay of standards*

Anti-inflammatory drug	**Concentration of the solution used in assay (mM)**	**Inhibition (%)**	**IC_{50} (µmol)**
Ibuprofen	0.5	35.51±0.15	<600
Flurbiprofen	0.5	36.17±0.24	<600
Aspirin	0.5	19.91±0.19	<600
Eserine	0.25	82.82±1.09	0.85±0.0001

Table 14: *Butyrylcholinesterase assay of prodrugs*

Prodrugs code	**Concentration of the solution used in assay (mM)**	**Inhibition (%)**	**IC_{50} (µmol)**
A1	0.5	77.57±0.19	78.81±0.01
A2	0.5	79.81±0.26	67.23±0.11
A3	0.5	65.88±0.36	214.41±0.05
A4	0.5	53.67±0.77	301.21±0.17
A5	0.5	76.18±0.25	118.91±0.08
A6	0.5	78.49±0.17	75.41±0.16
A7	0.5	78.53±0.39	73.61±0.22
A8	0.5	58.69±0.45	269.91±0.21
A9	0.5	61.25±0.85	238.51±0.11
A10	0.5	46.57±0.35	<500
A11	0.5	68.11±0.15	164.11±0.51
A12	0.5	69.57±0.33	149.11±0.18
A13	0.5	73.13±0.28	142.11±0.22
A14	0.5	69.71±0.45	156.71±0.18
A15	0.5	57.59±0.68	288.21±0.11
A16	0.5	54.83±0.21	<400
A17	0.5	63.15±0.33	221.61±0.14
A18	0.5	57.81±0.55	281.51±0.16
A20	0.5	84.24±0.32	45.11±0.21
A24	0.5	78.46±0.62	126.11±0.16
A25	0.5	68.11±0.21	142.71±0.15

DPPH Activity

In our results inhibition (%) by A4 (55.65) was highest in all the reactants and prodrugs, while A9 revealed the lowest % scavenging value (5.67). The assay regarding reduction capability of DPPH radicals on reacting with prodrugs was determined as a function of their concentration by the decrease in absorbance at 517 nm (DPPH). There is significant decrease in the concentration of DPPH radical due to the scavenging ability of prodrug solution. Table shows a sharp fall in the absorbance of DPPH in the prodrug A3, A4, A5, A6, A7, A11, A12, A13, A15, A17, A18, A20, A24, and A25. In comparison of prodrugs of ibuprofen with ibuprofen, A3, A4, A11, A12, and A18 showed enhanced performance than the parent drug. A6, A7, A8, A13, A15, A16, and A17 exhibited improved results than the parent drug flurbiprofen. All the aspirin's prodrugs (A20, A24, and A25) were better in inhibition than aspirin (Table 15 and Table 16).

Table 15: *DPPH activity of standards*

***Anti*-inflammatory drug**	**Concentration of the solution used in assay (mM)**	**Inhibition (%)**	**IC_{50} (µmol)**
Ibuprofen	0.5	11.33±0.11	<500
Flurbiprofen	0.5	6.37±0.22	<500
Aspirin	0.5	10.87±0.17	<500
Quercetin	0.25	93.21±0.97	16.96±0.14

Table 16: *DPPH activity of prodrugs*

Prodrug code	**Concentration of the solution used in assay (mM)**	**Inhibition (%)**	**IC_{50} (µmol)**
A1	0.5	7.92±0.39	<500
A2	0.5	6.57±0.92	<500
A3	0.5	20.81±0.48	<500
A4	0.5	55.65±0.38	<500
A5	0.5	10.59±0.22	<500
A6	0.5	15.15± 0.87	<500
A7	0.5	12.85±0.98	<500
A8	0.5	8.66±0.56	<500
A9	0.5	5.67±0.89	<500
A10	0.5	7.64±0.37	<500
A11	0.5	12.48±0.11	<500
A12	0.5	16.38±0.67	<500
A13	0.5	11.62±0.27	<500
A14	0.5	6.03±0.16	<500
A15	0.5	17.11±0.58	<500
A16	0.5	13.55±0.62	<500
A17	0.5	23.69±0.37	<500
A18	0.5	16.87±0.29	<500
A20	0.5	14.49±0.55	<500
A24	0.5	14.16±0.66	<500
A25	0.5	14.37±0.31	<500

3.2.3. Anti-tuberculosis activity

For getting the results for anti-tuberculosis activity, slants were observed carefully after 6 weeks of incubation according to the guide lines by W.H.O. Relative growth of an inoculum on a drug free controlled medium was compared (Figure 3) with any growth on culture media containing isoniazid, A4 and A9 in Figure 4, Figure 5 and Figure 6 respectively. No bacterial colonies were grown and observed on the culture media in the tubes containing the drugs while a good number of colonies were observed in drug free tube. It clearly indicated that new prodrugs synthesized have positive anti-tuberculosis activity. Mycobacterium tuberculosis bacteria were sensitive towards isoniazid and both the mutual prodrugs.

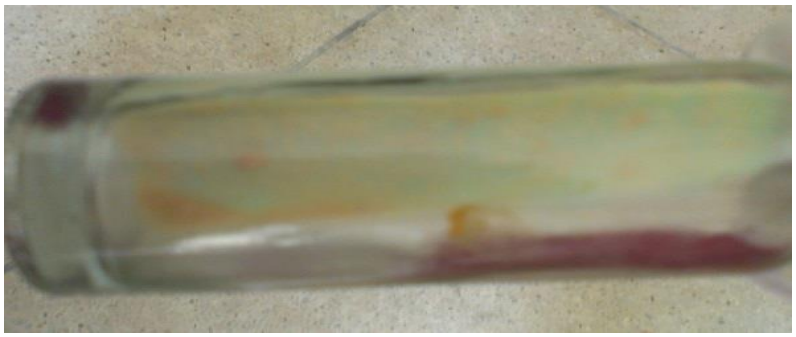


Figure 3: Mycobacterial colonies in drug free tube

Figure 4: No Mycobacterial colonies in the tube containing isoniazid

Figure 5: No Mycobacterial colonies in the tube containing A4

Figure 6: No Mycobacterial colonies in the tube containing A9

Table 17: *Anti-tuberculosis activity of the standard & prodrug*

Obs. no.	**Compounds**	**Anti-tuberculosis activity**
1	Isoniazid	+ve
2	A4	+ve
3	A9	+ve

3.2.4. Toxicity

Acute toxicity

For observing any possible toxic effects of synthesized mutual prodrugs on mice, these were carefully looked after and monitored, after giving dose, for 14 days. According to the OCED guidelines [120], some very important points were very carefully monitored, as these were important for deciding the acute toxicity of the drugs and prodrugs. These points are listed in the table 18.

Table 18: *Important points regarding toxic effects in mice*

Important factors regarding toxicity	**Responses**
Condition of the fur	Found normal
Skin	Found normal
Subcutaneous swellings	No swelling
Abdominal distension	Nil
Eyes dullness	Nil
Eyes opacities	Normal
Pupil diameter	Nil
Ptosis	Normal
Colour and consistency of the faeces	Normal
Wetness or soiling of the perineum	No
Condition of teeth	Found normal
Breathing abnormalities	Found normal
Gait	Found normal
Behaviour pattern	Found normal
Sleep	Slightly disturbed first day and then remained normal for rest of 13 days

Mice showed anxiety and distress after dosing for first half an hour but they got normal within first 4 hours after dosing. It was recorded that all these physical characters were detected to be normal in rest of all the days. All these above mentioned Conditions were observed for the signs of any possible toxicity after giving dose to mice, but there were no such symptoms. No toxic effects were observed in mice.

Mortality was the key criteria in assessing the acute toxicity (LD_{50}) of any drug. In 14 days there was no mortality recorded. The administration of the prodrugs did not show any significant changes in the body weight, indicating that it did not have adverse effects on body weight.

Sub-chronic toxicity test

Mice were observed carefully for the first seven days periodically twice a day and then on daily basis at the same time. Clinical observations were done prior to the first dose and then on weekly basis. There was slow but constant increase in the body weight during these 90 days indicated prodrugs did not produce any undesirable effects on the physiology of the mice. Consumption of food and water was slightly decreased during last 30 days. At the end of 90 days administration of drugs, the liver and stomach tissues were removed from mice and then were stored in 10% formalin. 5μm thick paraffin sections of these tissues were prepared stained with haematoxylin and eosin dye. These sections were studied for histopathological changes. The control group showed normal cellular architecture with well-presented cytoplasm and intact nucleus (Figure 7). The liver sections of drug treated mice (group II) showed hepatic cells with variable toxicity characterized by different levels of disarrangement and degeneration of hepatic cells. Mouse treated with ibuprofen showed granular cytoplasm along with cellular swelling. Nucleus also showed fragmentations (Figure 8). Mouse treated with flurbiprofen showed shrunken hepatic cords. Sinusoidal spaces

have become increased (Figure 9). Mouse treated with aspirin showed cellular swelling along with hydropic degeneration (Figure 10). Mouse treated with metronidazole cellular swelling along with change in cytoplasmic components (Figure 11). In sulfanilamide treated mouse granular cytoplasmic with increased sinusoidal spaces are visible (Figure 12). Mouse treated with sulfamethoxazole hydropic degeneration where nucleus was seen in normal condition (Figure 13).

The liver sections of mouse treated with prodrugs (group-III) showed lesser disarrangements and degeneration of hepatocytes. Sinusoidal spaces have sinusoidal capillaries and have few RBCs. The hepatocytes are less swollen and have some vacuoles. In some areas central veins can be seen to be congested. Nucleus can be seen normal in all these slides although very slight cytoplasmic swelling can be seen in some places (Fig 14-20).

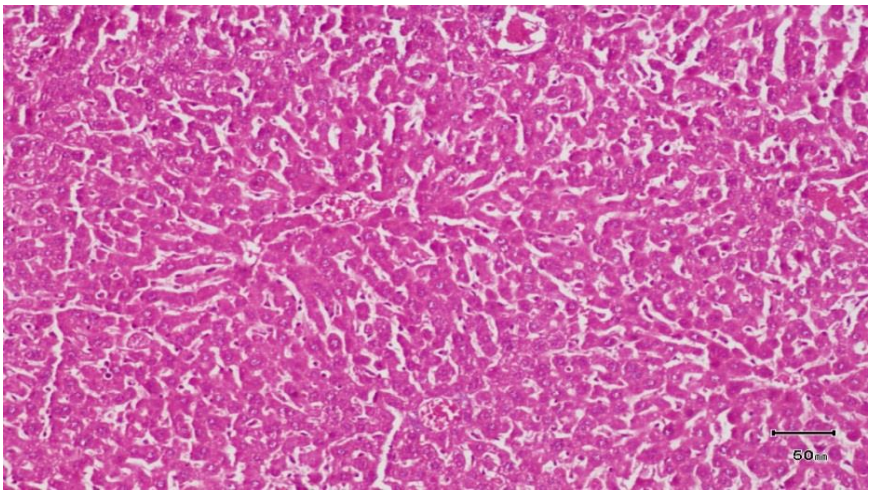


Figure 7*:* Section of the liver of the mouse of control group

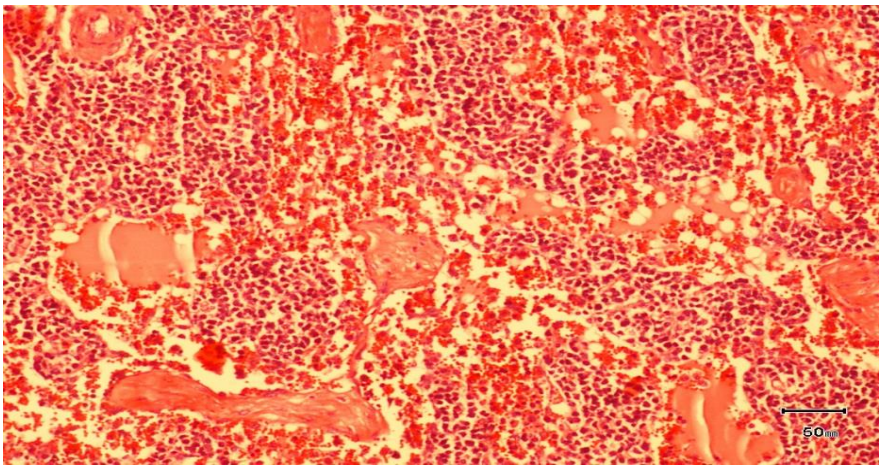


Figure 8: Section of the liver of mouse treated with ibuprofen

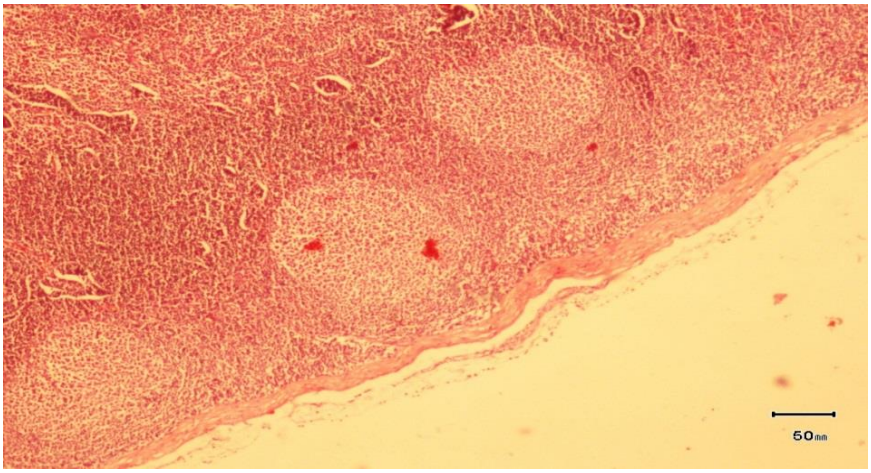


Figure 9: Section of the liver of the mouse treated with Flurbiprofen

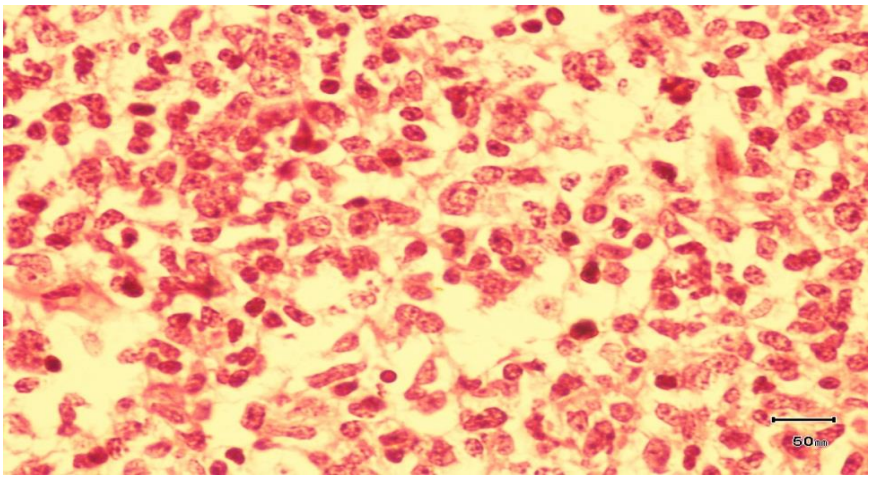


Figure 10: Section of the liver of the mouse treated with aspirin

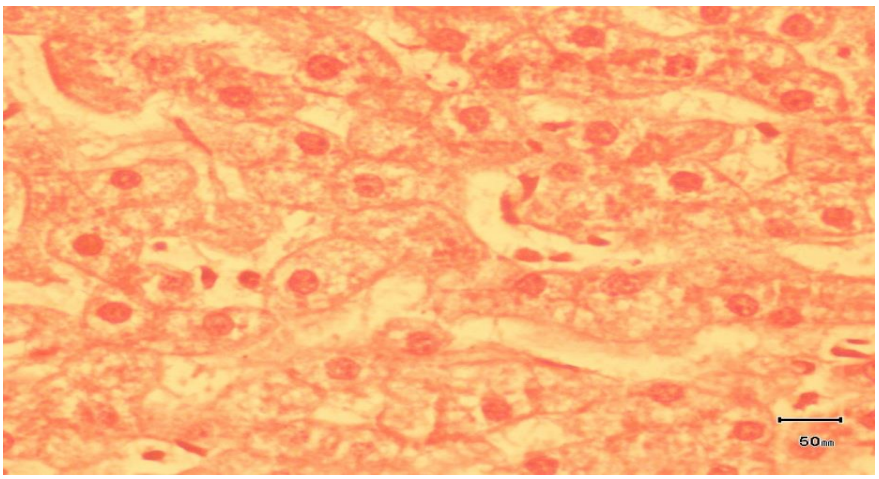


Figure 11: Section of the liver of moue treated with metronidazole

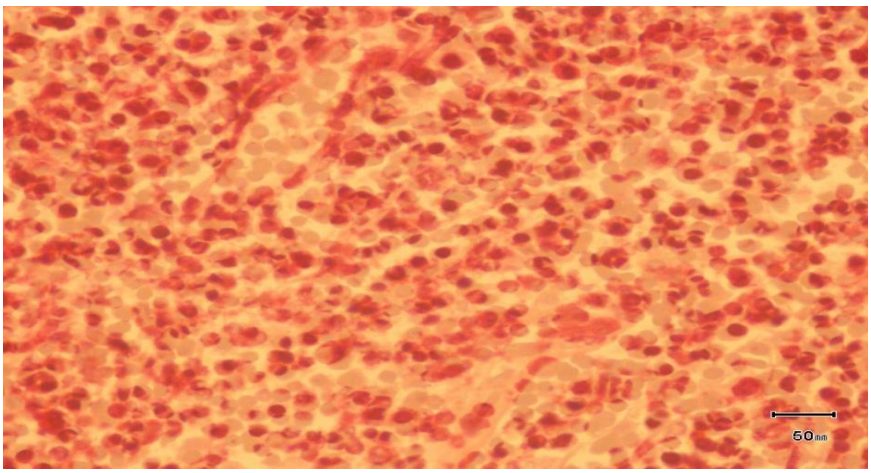


Figure 12: Section of the liver of mouse treated with sulfanilamide

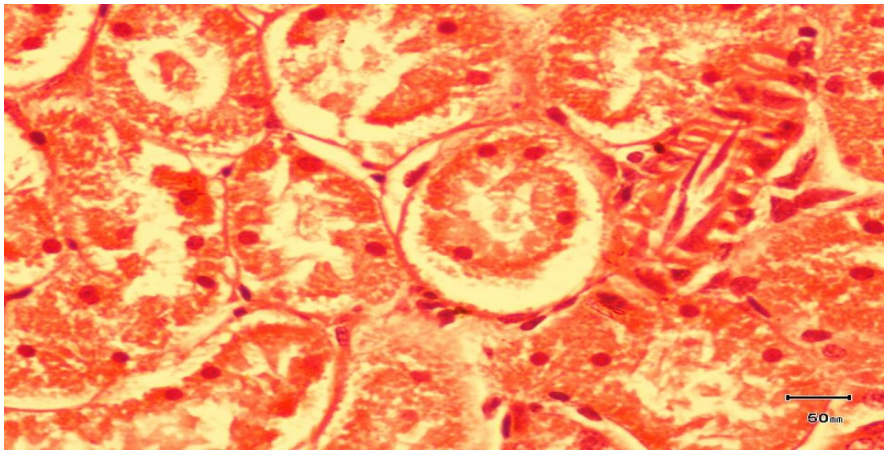


Figure 132: Section of the liver of the mouse treated with sulfamethoxazole

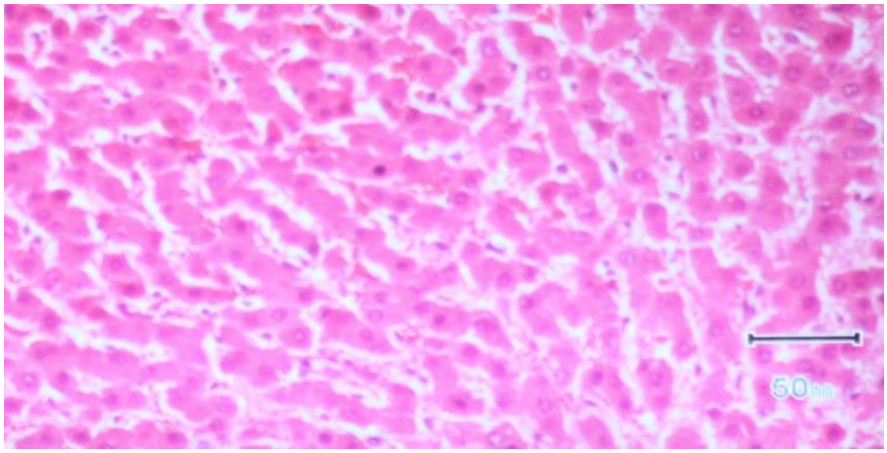


Figure 14: Section of the liver of the mouse treated with A5

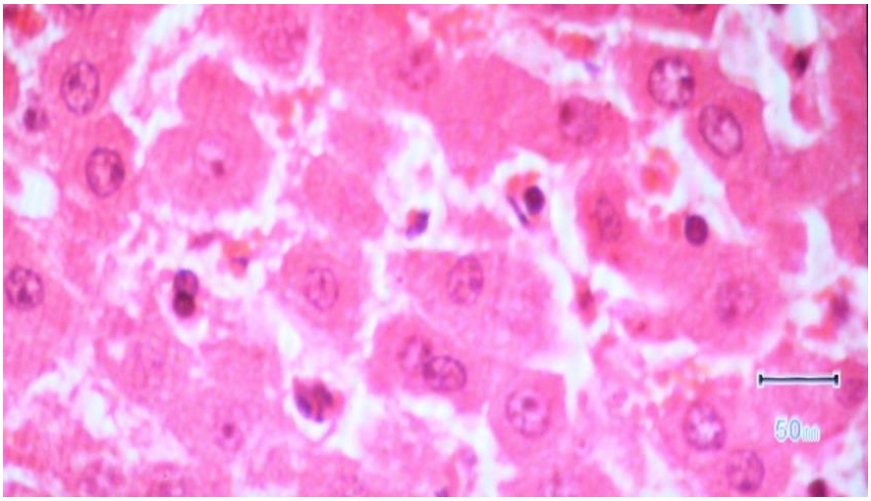


Figure 15: Section of the liver of the mouse treated with A6

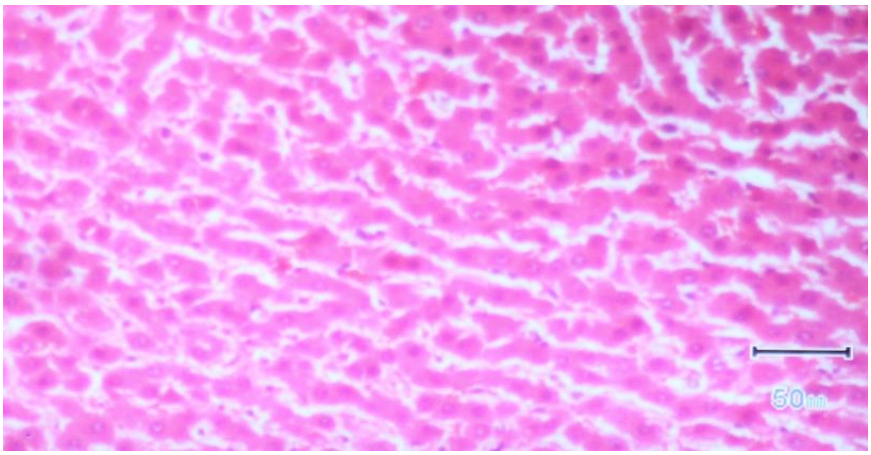


Figure 16: Section of the liver of the mouse treated with A11

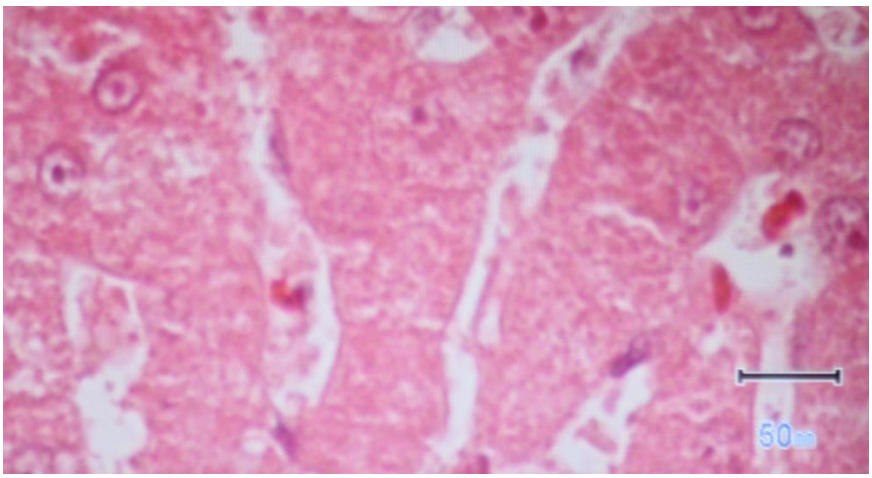


Figure 17: Section of the liver of the mouse treated with A13

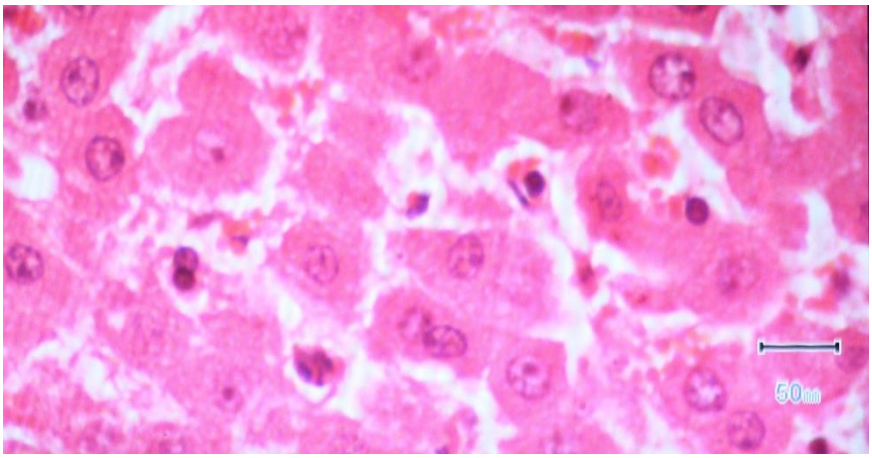


Figure 18: Section of the liver of the mouse treated with A14

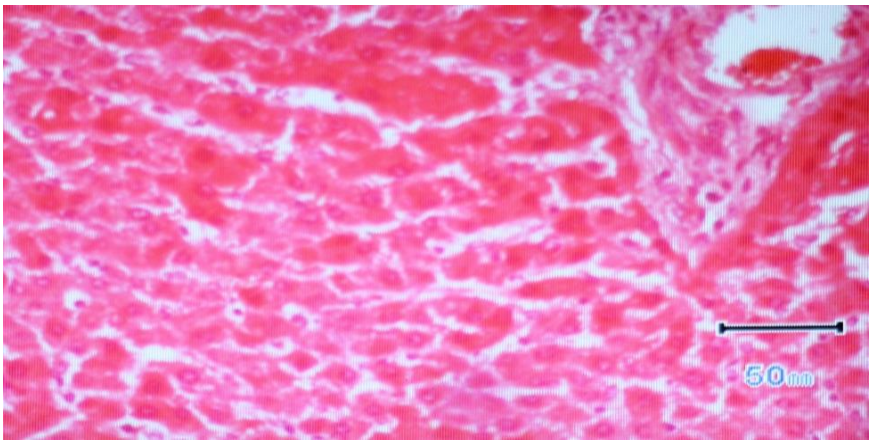


Figure 19: Section of the liver of the mouse treated with A20

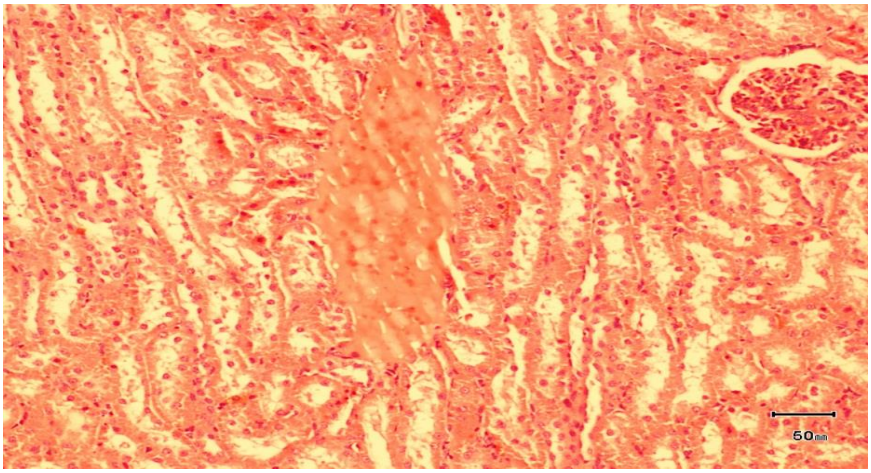


Figure 20: Section of the liver of the mouse treated with A24

3.3. Computational studies

Some of the physico-chemical properties like molar refractivity, molar volume, parachor, index of refraction, surface tension, density and polarizability were predicted using ACD/ilabs2. By using these predictions, a good set of database regarding compound was managed. These calculations were done for facilitating the further experimental designs by comparing the actual results with predicted ones. Structure related properties were also determined to help in quantitative structure activity relationship (Table 19 and Table 20). These in computational studies also

helped to predict the behaviour of the drug in vivo, for example Log BB, Log PS and bioavailability values (Table 21). One advantage of using these in computational studies was the detail about structure, physical, chemical and biological properties which were not possible. These reliable predictions results in increased speed of experiments. Another advantage of these studies is the reduction in animal and reagent use.

Table 19: *Computational predicted physico-chemical properties of the synthesized prodrugs*

Prodrug	**Molar refractivity (cm^3)**	**Molar volume (cm^3)**	**Parachor (cm^3)**	**Index of refraction**	**Surface tension (dyne/cm)**	**Density (g/cm^3)**	**Polarizability ($\times 10^{-24}$ cm^3)**
A1	147.16±0.4	412.3±5.0	1154.8±0.03	1.632±0.03	61.5±5.0	1.30±0.1	58.34±0.5
A2	129.61±0.4	374.7±3.0	133.1±6.0	1.608±0.02	57.7±3.0	1.244±0.036	51.38±0.5
A3	100.02±0.4	300.0±3.0	793.1±6.0	1.581±0.02	48.7±3.0	1.201±0.06	39.65±0.5
A4	94.12±0.3	289.6±3.0	751.5±4.0	1.563±0.02	45.3±3.0	1.123±0.06	37.31±0.5
A5	119.67±0.4	348.0±3.0	954.5±6.0	1.603±0.02	56.5±3.0	1.268±0.06	47.44±0.5
A6	106.35±0.4	303.3±3.0	816.0±6.0	1.618±0.02	52.3±3.0	1.313±0.06	42.16±0.5
A7	131.31±0.4	361.8±3.0	1017.7±6.0	1.645±0.02	62.6±3.0	1.355±0.06	52.05±0.5
A8	129.61±0.4	374.7±3.0	1033.1±6.0	1.608±0.02	57.7±3.0	1.244±0.06	51.38±0.5
A9	99.93±0.3	292.8±3.0	778.4±4.0	1.598±0.02	49.8±3.0	1.240±0.06	39.61±0.5s
A10	124.98±0.4	358.5±3.0	994.8±6.0	1.614±0.02	59.2±3.0	1.262±0.06	49.54±0.5
A11	99.18±0.5	302.5±7.0	772.4±8.0	1.569±0.05	42.5±7.0	1.18±0.1	39.31±0.5

A12	108.91±0.4	308.8±5.0	855.3±6.0	1.623±0.03	58.8±5.0	1.30±0.1	43.17±0.5
A13	106.09±0.5	310.7±7.0	810.0±8.0	1.598±0.05	46.2±7.0	1.27±0.1	42.05±0.5
A14	125.99±0.4	351.3±3.0	977.4±6.0	1.636±0.02	59.8±3.0	1.364±0.06	49.94±0.5
A15	153.49±0.4	410.0±5.0	1177.8±6.0	1.671±0.03	68.0±5.0	1.40±0.1	60.84±0.5
A16	115.23±0.4	306.7±5.0	878.3±6.0	1.674±0.03	67.2±5.0	1.43±0.1	45.68±0.5
A17	119.63±0.4	318.1±5.0	907.5±6.0	1.675±0.03	66.2±5.0	1.42±0.1	47.42±0.5
A18	113.31±0.4	320.2±5.0	884.6±6.0	1.625±0.03	58.2±5.0	1.29±0.1	44.92±0.5
A20	83.40±0.4	293.3±3.0	662.4±6.0	1.613±0.02	58.6±3.0	1.396±0.06	33.06±0.5
A24	103.05±0.4	287.3±3.0	823.8±6.0	1.636±0.02	67.5±3.0	1.445±0.06	40.85±0.5
A25	108.36±0.4	297.7±3.0	864.1±6.0	1.648±0.02	70.9±3.0	1.432±0.06	42.96±0.5

Table 20: *Computational predicted Structure related properties*

Prodrug name	No. of hydrogen bond donors	No. of hydrogen bond acceptor	TPSA	No of rotatable bonds
A1	3	8	141.11	9
A2	2	7	109.43	8
A3	3	5	97.64	6
A4	2	5	71.09	6
A5	2	7	109.68	8
A6	3	5	97.64	5
A7	2	7	109.43	7
A8	2	7	109.43	8
A9	2	5	71.09	5
A10	2	7	109.43	8
A11	0	7	92.95	9
A12	2	6	112.01	6
A13	0	7	92.95	8
A14	2	7	109.68	7
A15	3	8	141.11	8
A16	2	6	112.01	5
A17	2	6	112.01	6
A18	2	6	112.01	7
A20	3	7	123.94	5
A24	2	9	135.98	7
A25	2	9	135.73	7

Table 21: *Computational predicted LogBB, LogPS and Bioavailability of A1-A25 .*

Prodrug	Log BB	LogPS	Bioavailability
A1	0.44	-2.8	Less than 30%
A2	-1.8	0.28	Between 30-70%
A3	0.17	-2.5	More than 70%
A4	0.66	-1.7	More than 70%
A5	0.17	-2.4	Between 30-70%
A6	-1.04	-1.7	More than 70%
A7	-0.94	-1.9	Between 30-70%
A8	-0.94	-2.5	Between 30-70%
A9	0.66	-2.5	More than 70%
A10	-0.07	-1.8	Between 30-70%
A11	1.18	-1.2	Less than 30%
A12	-1.05	-2.8	Between 30-70%
A13	-0.58	-1.1	Less than 30%
A14	-1.04	-2.5	Between 30-70%
A15	-0.03	-2.7	Less than 30%
A16	0.04	-2.6	Between 30-70%
A17	-0.09	-	Less than 30%
A18	0.13	-2.7	Between 30-70%
A20	-1.04	-2.3	Between 30-70%
A24	-1.07	-3.4	Between 30-70%
A25	-1.02	-2.3	Between 30-70%

Lipinski's rule of five (also known as Pfizer's rule of five) is a basic criteria to evaluate druglikeness which determines if a chemical compound can be used as orally active drug in humans. According to the rule [127] a drug molecule should not have more than 5 hydrogen bond donor and also not more than 10 hydrogen bond acceptors. All the synthesized prodrugs follow these two criteria. Third criteria are that molecular mass should not be greater than 500 daltons. All the synthesized prodrugs except A1 and A15 followed this as well. Molar refractivity should be between 40 to130. Prodrugs except A1, A15 followed this. Polar surface area of the prodrugs was less than 140 $Å^2$ except A1 and A15. Bioavailability of majority of the synthesized prodrugs was greater than 70%. A drug having 10 or lesser rotatable bonds and polar surface area lesser than 140 $Å^2$ are predicted to have good oral bioavailability [128]. Compounds having more than more than 0 value of log BB are CNS active compounds [129]. Nearly all the compounds except A1, A3, A4, A5, A9, A11, A16 are CNS inactive so these are safe to use as these will not penetrate through the blood brain barrier (Table 22).

Table 22: *Computational predicted LD_{50} values (mg/kg) of prodrugs*

Prodrug	Mouse				Rat	
	Intraperitoneal (IP)	Oral	Intravenous	Subcutaneous	Intraperitoneal	Oral
A2	770	17000	350	1400	960	11000
A3	610	8600	130	1800	1200	5000
A4	530	1100	160	650	250	1000
A5	870	4900	330	1600	2000	11000
A6	950	5100	130	1700	1200	2000
A7	1000	8300	340	2400	1300	6900
A8	770	13000	350	1400	960	11000
A9	1000	590	100	1000	290	610
A10	810	17000	380	1500	1100	11000
A11	790	1900	140	2200	1800	1900
A12	2300	13000	1600	3400	3100	13000
A13	1100	1600	130	2600	1900	880
A14	1100	4000	270	2100	2200	5900
A16	3700	9000	1300	4100	3000	5500
A17	3500	8200	1100	3900	3200	4200
A18	2300	11000	1300	5200	3500	9900
A20	1100	12000	340	2200	1600	5700
A24	2200	6300	680	1600	3500	7900
A25	1400	16000	600	1700	1800	7800

3.4. Conclusions

The mutual prodrugs were successfully synthesized from anti-infectives (ampicillin, isoniazid, metronidazole, sulfamethoxazole, sulfamerazine, sulfamethazine, sulfanilamide, 7-ADCA, 7-AVCA, Cephazolin) and NSAIDs (ibuprofen, flurbiprofen, aspirin, benzydamine). These prodrugs were completely characterized by using various analytical techniques including electronic spectroscopy, FT-IR, ^{1}H NMR and ^{13}C NMR and single crystal structure determination by XRD. The prodrugs were found to be more active and less toxic than the parent drugs in various *in vitro* and *in vivo* tests. In this work, it has been demonstrated that NSAIDs containing carboxylic groups can easily be covalently coupled with anti-infectives containing amino groups to produce mutual prodrugs with dual activity and improved toxicity profile.

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